Saskatchewan

A D F

Agriculture Development Fund

FINAL REPORT

19970334

USE OF TRADITIONAL CHINESE HERB MEDICINES IN STUDY OF SASKATCHEWAN WILD PLANTS FOR NATURAL ANTICANCER SUBSTANCES

Funded by
The Agriculture Development Fund

November 2001

Prepared by: University of Saskatchewan

SAF
Saskatchewan Agriculture & Food
Adding value to agriculture...through research and development

102-05956/

FINAL REPORT

Use of traditional Chinese herb medicines in study of Saskatchewan wild plants for natural anticancer substances

ADF Project No. 97000334

Branka Barl[1] and Robert A. Hickie[2]

[1]Department of Plant Sciences and [2]Department of Pharmacology
University of Saskatchewan

April 2001

TABLE OF CONTENTS

A. ABSTRACT
B. EXECUTIVE SUMMARY
C. TECHNICAL REPORT
1. Introduction
2. Plant Collection
2.1 Materials and Methods
2.2 Results
3. Plant Extraction and Screening of Extracts for Anticancer Properties *in vitro*
3.1 Plant Extraction
Materials and Methods
Results
3.2 Screening of Extracts for Anticancer Properties *in vitro*
Materials and Methods
Results
4. Fractionation of Promising Extracts using Bioassay-guided Fractionation Strategy
4.1 *Xanthium strumarium* (cocklebur)
4.2 *Echinacea angustifolia* (narrow-leaved purple coneflower)
APPENDIX A List of plants with potential anticancer activity collected in the wild throughout Saskatchewan over two years, 1999 and 2000.
APPENDIX B Images of 40 plant species used for testing in this study.

LIST OF TABLES

Table1 . List of plant species from Saskatchewan used for the screening study.
Table 2. Percentage mortality of the most active fractions against MDA-MB-231 (Human Breast Cancer Cell Line).
Table 3. Percentage mortality of the most active fractions against WiDr (Human Colon Cancer Cell line).
Table 4. Percentage mortality of the most active fractions against NCI-417 (Human Lung Cancer Cell line)
Table 5. Cytotoxic activity of plant parts of tansy (*Tanacetum vulgare*) expressed as percentage mortality of three human cancer cell lines (colon, breast and lung).

LIST OF FIGURES

Fig. 1 Selected protocol for plant extraction using solvents of increasing polarity.
Fig. 2 CellTiter 96 Assay flowchart.
Fig. 3. Molecular structure of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and its corresponding reaction product formazan.
Fig. 4. Mortality rate of human colon cancer cell line WiDr treated with plant extracts.
Fig. 5. Mortality rate of human breast cancer cell line MDA-MB-231 treated with plant extracts.
Fig. 6. Mortality rate of human lung cancer cell line NCI-417 treated with plant extracts
Fig. 7-12. HPLC profiles of various active fractions from *Echinacea angustifolia* and *Xanthium strumarium*

A. Abstract

Anticancer drugs of plant origin have been used in modern medicine and traditional medicines of many cultures around the world. In Traditional Chinese Medicine (TCM) over 1000 plants are used as a source of bioactive agents to treat various forms of cancer. In this collaborative study between the Department of Plant Sciences and Department of Pharmacology, fourthy species from 17 plant families, of which 19 species were from the family Compositae were collected throughout Saskatchewan from May to October over two years and screened for the cytotoxic activity. About half of the plants studied were used in TCM, while the remaining half were related species from the same genera as TCM plants, with the exception of *Echinacea angustifolia*, plant native to Saskatchewan and not used in TCM. Plant parts of interest were extracted in methanol, and crude methanol extract partitioned between solvents of increasing polarity such as hexane, chloroform, methanol and water, using a modified protocol of the National Cancer Institute. Methanol-insoluble residue was extracted in boiling water in following TCM tradition. The resulting 200 extracts, 5 per each species, were tested for *in vitro* cytotoxicity at a single concentration of 100 µg/mL against human colon (WiDr ATCC), breast (MDA-MB-231 ATCC), and lung carcinoma (NCI-417) using the Cell Titer 96™ Non-Radioactive Cell Proliferation Assay (MTT method). Hexane and chloroform extracts from 12 species caused >95% mortality of all three cancer cells lines tested with IC_{50} values < 20 µg/mL. The most active were roots of *Lithospermum incisum, Alisma plantago-aquatica* and *Echinacea angustifolia,* burrs of *Xanthium strumarium,* and entire plant of *Matricaria matricarioides.* This demonstrated that ethnopharmacological screening is a useful tool in the quest for discovering new bioactive agents. The bioactivity-directed fractionation of most active *X. strumarium* fraction using silica-gel chromatography and semi-preparative HPLC resulted in isolation of a sesquiterpene lactone-like compound, whose structure elucidation by spectroscopic methods is underway.

B. Executive Summary

This is the final report for the two-year ADF Project #97000334 which commenced in March 1999. The objectives of this project are:

1. to collect and botanically identify wild plants across Saskatchewan that based on Chinese traditional experiences may have anticancer properties;
2. to prepare extracts and screen extracts for anticancer properties *in vitro;*
3. to select extracts with promising anticancer activities and fractionate them using bioassay-guided fractionation strategy;
4. to interact with pharmaceutical and other industries in Canada and elsewhere for further studies that could lead to commercialization of most promising plant extracts or natural compounds thereof.

Given that plants are used as source of anticancer drugs in modern medicine (e.g. taxol from Pacific yew tree, vinblastine from Madagascar periwinkle) and traditional medicines of many cultures around the world, we have initiated this collaborative study with the Department of Pharmacology (Dr. Hickie) on potential cancer chemotherapeutic agents from Saskatchewan wild plants. An ethnopharmacological approach, namely the knowledge of traditional Chinese medicine was applied as a main tool in selecting study material from over 1,000 native and introduced species growing in the province.

Research activities over the past 6 months covered first two objectives and concentrated in three areas: (1) field collection and post-collection processing of plant material selected for the project (drying and storing for further studies, and pressing and mounting for deposition of voucher specimens in W.P. Fraser Herbarium); (2) initiation of plant material extraction and extract separation into hexane, chloroform, methanol and aqueous fraction; (3) initiation of testing of generated fractions for anticancer activity *in vitro* against three human cancer cell lines using Standard CellTiter CellTiter 96™ Assay Protocol. Testing was conducted in the Saskatoon Cancer Centre Research Unit.

Total of 81 species were collected in the wild throughout Saskatchewan from June to middle of October, 1999. They were botanically identified with the assistance of Peggy Ann Ryan, W. P. Fraser Herbarium. Preparation of 72 herbarium speciemen was initiated. All plant material was dried at room temperature for 2-3 weeks following Chinese tradition until material was safe to store for further studies (moisture content<10%). First batch of 10 plant species: *Thalictrum venulosum, Rhus radicans, Lepidium apetalum, Artemisia absinthium, Leonurus cardiaca, Artemisia vulgaris, Aster brachyactis, Sonchus arvensis, Tanacetum vulgare* and *Echinacea*

angustifolia were extracted in methanol yielding crude methanol extract (11.2-22.2% of plant material) and plant residue. Crude methanolic extract was further fractionated by partitioning between solvents of increased polarity into hexane, chloroform, methanol and aqueous fractions according to the established extraction protocol. Plant residue was extracted by boiling water yielding 5th boiling water fraction for each of 10 plant species. Solvents from all extracts were removed under vacuum or by freeze drying (aqueous extracts) and dry extracts stored for further study. Testing of activity against colon carcinoma (WiDr ATTC) indicated that hexane fraction of tansy and absinthe aerial parts, chloroform fraction of veiny meadow-rue root and hexane fraction of motherwort aerial parts expressed the highest anticancer activity. They will be subjected to further testing. Hexane extract of narrow-leaved purple coneflower root also exhibited considerable cytotoxic activity. Results obtained to date are encouraging and project is progressing as planned.

Several Saskatchewan plants are promising in suppressing cancer cell proliferation in *in vitro* studies. The study in progress includes bioactivity-directed fractionation of the cocklebur chloroform extract and *E. angustifolia* hexane extract aimed at isolating and revealing structure of compounds believed to be responsible for antitumour activity. Potential benefits of the results of this research include the development of new nutraceutical products that can address the prevention of cancer, as opposed to its treatment.

C. Technical Report

1. Introduction

Drugs in TCM include formulae that are made from the roots, stems, bark, leaves, seeds or flowers of many plants, as well as some mineral and animal parts. TCM is a wealth of medical knowledge gained from over 4,000 years of observation, investigation and clinical experience. It has evolved as an empirical science and its theories and treatments have been repeatedly in use and were refined over this long period of time. From the ancient Medicinal monographs (since 206 BC), it is known that there are 1042 types of anticancer materials recorded in TCM. Despite these many important past contributions from the plant kingdom, a great many plant species have never been described and remain unknown to science, and relatively few have been surveyed systematically to any extent for biologically active chemical constituents. Thus, it is reasonable to expect that new plant sources of valuable and pharmaceutically interesting material remain to be discovered and developed.

Under the assumption that flora of Saskatchewan and northern China consist of some of the same plant species and/or related species from the same genera, Saskatchewan plant distribution maps and taxonomical records, and TCM records, were researched and compared. It appeared that at least 35 species out of estimated 2400 species found throughout Saskatchewan are used in TCM. Consequently, it became convincing that screening of Saskatchewan plants for potential sources of antineoplastic agents was a worthwhile effort.

In the field of natural product drug discovery, bioassay-directed fractionation is the most commonly used method. Plant extracts are prepared, tested in a bioassay systems, and extracts demonstrating a positive response (mortality rate of cancer cells>90%), are considered as active leads. The latter are fractionated, and each fraction is subjected to an evaluation in the bioassay test system, the most active fractions are further fractionated and this process continues until a pure active compound is isolated. The compound is then subjected to a series of analytical procedures aimed at elucidating the structure of the active lead.

We have used the ethnopharmacological approach in selected plant material for this study. Plant species were selected on the basis of information derived from Traditional Chinese Medicine.

Achievements

2. Plant Collection

Materials and Methods

1. to collect and botanically identify wild plants across Saskatchewan that based on Chinese traditional experiences may have anticancer properties;

All 40 plant species collected over two years, were taxonomically identified and voucher specimens deposited in the W.P. Fraser Herbarium, University of Saskatchewan. The plants were sorted, plant parts of interest were washed, sliced, air dried and stored under controlled temperature (10°C) and relative humidity (40-45%). Approximately 200 g of plant material was ground in a Model 4 Thomas-Wiley Laboratory Mill using a 2-mm sieve (20 mesh) prior to extraction. The extraction protocol is illustrated in Fig. 1.

With assistance of Gerald Ivanochko from Saskatchewan Agriculture and Food (Sustainable Production), Peggy Ann Ryan from W. P. Fraser Herbarium and Donna Dunlop, Herb Research Program, University of Saskatchewan, plant material was collected at various locations throughout Saskatchewan such as Saskatoon, Indian Head, Prince Albert, La Ronge, Outlook, Wakaw Lake, Bidulph, along Highway # 5, Highway #11, Highway # 41, and Highway #219. Up to now, 82 species were collected and taxonomically verified by the assistance of Peggy Ann Ryan (Table 1). Every plant material was given a collection number. 72 Herbarium specimens and 84 photos were made to document collected plant. Upon collection, desirable plant parts were separated, washed, air dried and kept in paper bags in controlled temperature storage (10°C) and at humidity < 50% until subjected for extraction.

Results

List of plant material collected from 83 species is provided in Appendix A. Images of 40 plant species used in actual testing for anti-tumor activity is provided in Appendix B.

Considering the time and funds limitation of this project, it was decided to direct our studies to 39 species of medicinal plants which belong to the genera *Artemisia, Aster, Carduus, Cirsium, Cormus, Senecio, Solidago, Taraxacum and Xanthium* in Composite Family; genus *Acorus* in Araceae Family; genus *Alisma* in Alimaceae Family; genera *Asparagus* and *Maianthemum* in Liliaceae Family; genera *Leonurus*, *Mentha* and *Scutellaria* in Labiatae Family; genus *Lepidium* in Cruciferae Family; genus *Lythrum* in Lythraceae Family; genera *Polygonum* and *Rumex* in Polygonaceae Family; genus *Rhus* in Anacardiaceae Family; genera *Anemone*, *Ranunculus* and *Thalictrum* in Ranunculaceae Family; genus *Apocynum* in Apocynaceae Family; genus *Aralia* in Araliaceae Family; genera *Dryopteris* and *Matteuccia* in Polypodiaceae Family; genus *Crataegus* in Rosaceae Family; genus *Equisetum* in Equisetaceae Family; genera *Glycyrrhiza* and *Meliotus* in Leguminosea Family; genus *Lonicera* in Caprifoliaceae Family; genus *Phragmites* in Gramineae and *Pinus* in Pinaceae Family.

3. Plant Extraction and Screening of Extracts for Anticancer Properties *in vitro*

3.1 Plant Extraction

Materials and Methods

Dry plant material was ground in Model 4 Thomas-Wiley Laboratory Mill using 20 mesh sieve. Typically, 100 g of plant material was extracted according to the protocol elaborated in Figure 1. From each plant species 5 fractions were generated. Phytochemical studies were conducted in the Herb Research Laboratory.

Table 1: List of plant species from Saskatchewan used for the screening study.

No.	Coll. I.D.	Species & Family	Common Name & Chinese Name	Plant Part Collected & Date Collected
1.	058	*Acorus calamus* Araceae (Arum Family)	Sweet flag Chang Pu	Rhizome & Root September 8
2.	075	*Alisma plantago-aquatica* Alismaceae (Arrowhead Family)	Common water-plantain Xiao Hua Ze Xie	Root October 8
3.	001	*Anemone patens* Ranunculaceae (Buttercup Family)	Crocus anemone Shen Ye Bai Tou Weng	Rhizome May 5
4.	003	*Aralia nudicaulis* Araliaceae (Ginseng Family)	Wild sarsaparilla Luo Jing Cong Mu	Stem & Rhizome June 4
5.	016	*Artemisia absinthium* Compositae (Sunflower Family)	Absinthe Ni Yin Chen Hao	Aerial parts May 28
6.	009	*Artemisia campestris* Compositae (Sunflower Family)	Plains wormwood Hong Jing Hao	Whole plant June 23
7.	008	*Artemisia frigida* Compositae (Sunflower Family)	Pasture sage Leng Hao, Bai Hao	Whole plant June 17
8.	021	*Artemisia ludoviciana* Compositae (Sunflower Family)	Prairie sage Lu De Wei Hao	Whole plant July 20
9.	043	*Artemisia vulgaris* Compositae (Sunflower Family)	Common wormwood Ye Ai (Ai Hao, Ai Ye)	Aerial parts August 19
10.	069	*Asparagus officinalis* Liliaceae (Lily Family)	Asparagus Shi Diao Bai	Rhizome September 17
11.	072	*Aster brachyactis* Compositae (Sunflower Family)	Rayless aster	Aerial parts September 17
12.	059	*Aster ciliolatus* Compositae (Sunflower Family)	Lindley's aster Zi Wan	Root September 8
13.	035	*Carduus nutans* Compositae (Sunflower Family)	Nodding thistle Fu Chui Fei Ji	Aerial parts July 28
14.	033	*Cirsium arvense* Compositae (Sunflower Family)	Canada thistle Tian Ji	Aerial parts July 19
15.	027	*Cladina stellaris*	Northern reindeer lichen	Whole plant June 23
16.	074	*Echinacea angustifolia* Compositae (Sunflower Family)	Purple coneflower Xia Ye Zi Zhuo Hua	Root October 6
17.	061	*Epilobium angustifolium* Onagraceae (Evening-primrose Family)	Fireweed Liu Lan	Root September 9
18.	025	*Equisetum sylvaticum* Equisetaceae (Horsetail Family)	Woodland horsetail Lin Wen Jing	Whole plant June 23
19.	036	*Helianthus petiolaris* Compositae (Sunflower Family)	Prairie sunflower Ping Yuan Xiang Ri Kui	Whole plant July 28
20.	083	*Inonutus obliquus* Polyporaceae	Chaga, Birch Mushroom Shu She	Whole mushroom September 28
21.	042	*Leonurus cardiaca* Labiatae (Mint Family)	Motherwort Ou Yi Mu Cao	Aerial parts August 19
22.	010	*Lepidium apetalum* Cruciferae (Mustard Family)	Common pepper-grass Ting Li Zi, Du Xing Cai	Seed July 20
23.	002	*Lithospermum incisum* Boraginaceae (Forget-me-not Family)	Narrow-leaved puccoon Zi Cao	Root June 3
24.	049	*Lycopodium obscurum* Lycopodiaceae (Clubmoss Family)	Ground-pine Yu Bai	Whole plant September 8

Table 1. List of plant species from Saskatchewan used for the screening study continued.

No.	Coll. I.D.	Species & Family	Common Name & Chinese Name	Plant Part Collected & Date Collected
25.	071	*Lythrum salicaria* Lythraceae (Loosestrife Family)	Purple loosestrife Qian Qu Cai	Aerial parts September 17
26.	082	*Matricaria matricarioides* Compositae (Sunflower Family)	Pineappleweed	Whole plant September 15
27.	018	*Matteuccia struthiopteris* Polypodiaceae (Fern Family)	Ostrich fern Xiao Ye Guan Zhong	Rhizome June 23
28.	029	*Melilotus alba* Leguminosae (Pea Family)	White sweet-clover Bai Xiang Cao Mu Xi	Stem&Root July 2
29.	041	*Mentha arvensis* Labiatae (Mint Family)	Wild mint Bo He	Aerial parts August 18
30	076	*Polygonum amphibium* Polygonaceae (Buckwheat Family)	Water smartweed Liang Xi Liao	Root, stem & leaves, October 8
31.	005	*Rhus radicans* Anacardiaceae (Cashew Family)	Poison-ivy Qi Gen Du Teng	Root June 4
32.	031	*Rumex fennicus* Polygonaceae (Buckwheat Family)	Field dock Hong Niu Er Da Huang	Root July 2
33.	023	*Senecio vulgaris* Compositae (Sunflower Family)	Common groundsel Ou Zhou Qian Li Guang	Whole plant June 23
34.	037	*Solidago missouriensis* Compositae (Sunflower Family)	Low goldenrod Yi Zhi Huang Hua	Whole plant July 28
35.	064	*Solidago mollis* Compositae (Sunflower Family)	Velvety goldenrod Yi Zhi Huang Hua	Whole plant September 3
36.	065	*Sonchus arvensis* Compositae (Sunflower Family)	Perennial sow-thistle Qu Mai Cai	Whole plant September 16
37.	070	*Tanacetum vulgare* Compositae (Sunflower Family)	Tansy Ju Hao (Ai Ju)	Aerial parts September 17
38.	017	*Taraxacum officinale* Compositae (Sunflower Family)	Dandelion Ou Zhou Pu Gong Ying	Aerial parts & roots May 27
39.	004	Thalictrum venulosum Ranunculaceae (Buttercup Family)	Veiny meadow-rue Wang Zh. Tang Song Cao	Root&Aerial parts June 14
40.	047	*Xanthium strumarium* Compositae (Sunflower Family)	Cocklebur Cang Er	Mature fruit September 4

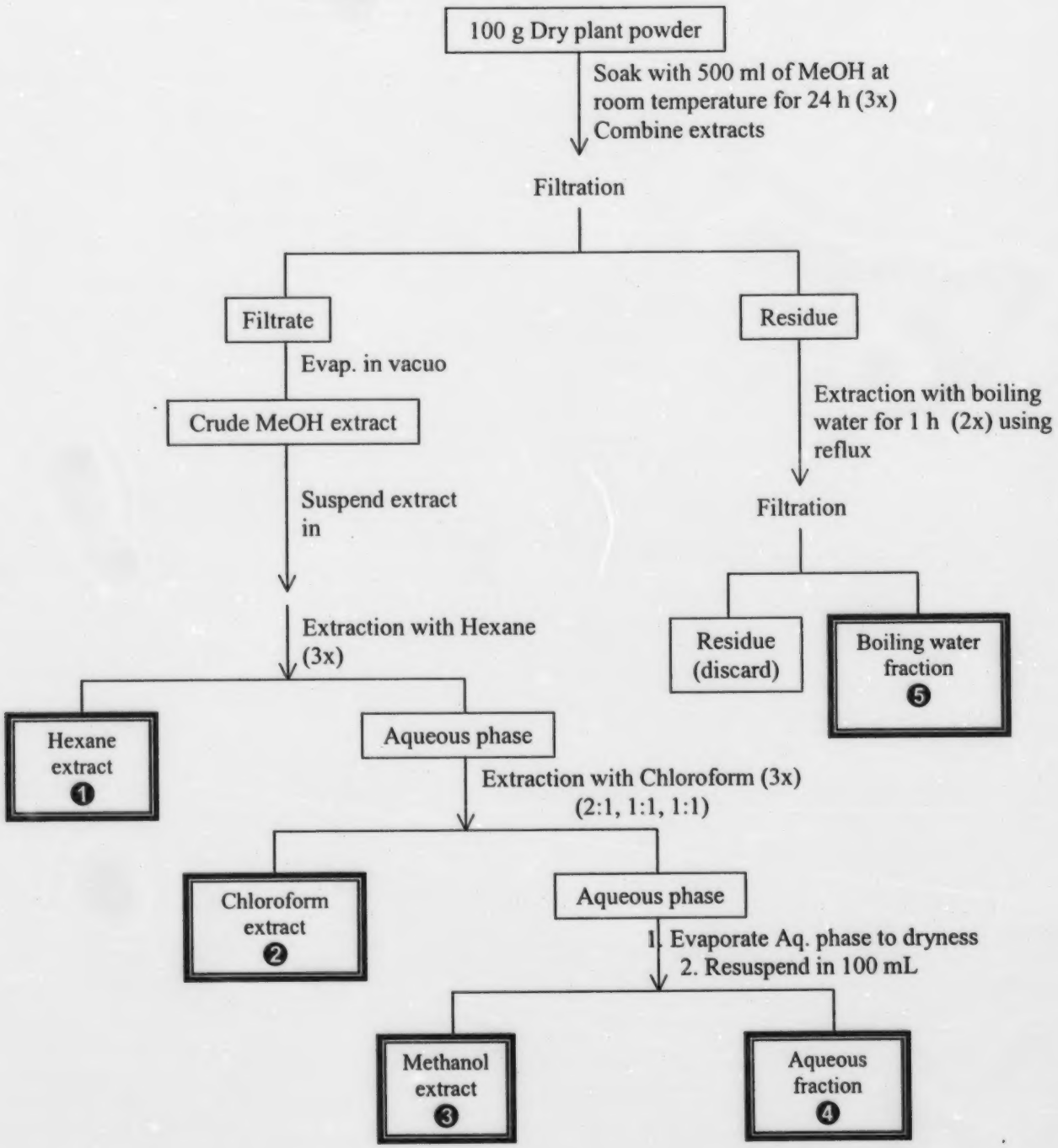


Figure 1. Selected protocol for plant extraction using solvents of increasing polarity.

Results

The yield of crude methanol extract for the 15 species tested was in the range of 10.2 to 24.2 % of plant material. Upon partitioning of crude methanol extract between hexane, chloroform, methanol and water, each of the 75 extracts (Table 2) generated was subjected to cytotoxicity determination. Extracts that caused cancer cell mortality >75-80% in comparison to untreated cells were selected for multidose testing in order to calculate IC_{50} values. In total, thirty, twenty-nine and thirty-six fractions were tested against WiDr, MDA-MB-231 and NCI-417 cell lines, respectively. The IC_{50} values of selected active extracts were in the range of 3.3-70.4 µg/mL, 2.4-143.3 µg/mL, and 0.02-235.1 µg/mL, respectively. A plant extract is regarded active if it shows an IC_{50} value < 20 µg/mL. The $CHCl_3$ extracts from *Xanthium strumarium* (cocklebur) and *Tanacetum vulgare* (tansy) exhibited significant cytotoxic response in all three cell lines tested. The IC_{50} values were 3.27 and 9.13 µg/mL against WiDr, 6.24 and 5.56 µg/mL against MDA-MB-231 and 0.02 and 2.37 µg/mL against NCI-417, respectively.

The hexane extract from *Echinacea angustifolia* (narrow-leaved purple coneflower) and the $CHCl_3$ extract from *Carduus nutans* (nodding thistle) showed IC_{50} values of 3.97 and 9.25 µg/mL against NCI-417, respectively. All other extracts showed IC_{50} values above 10 µg/mL and are considered to exert a general moderate cytotoxic activity. Overall, the MeOH and both aqueous extracts exhibited low cytotoxicity, if any. The MDA-MB-231 appeared to be the least susceptible cell line of the three cell lines tested.

Table 2. Yield of crude methanol extract and various fractions from plant species used in the screening study.

Plant Species	Crude Methanol Extract (g)	Hexane Fraction (g)	Chloroform Fraction (g)	Methanol Fraction (g)	Aqueous Fraction (g)	Recovery (g)	Boiling Water Fraction (g)
Artemisia campestris	13.9	2.0	0.6	9.7	0.9	13.2	17.4
Artemisia ludoviciana	18.1	2.0	0.8	13.8	0.7	17.3	12.6
Artemisia vulgaris	10.2	2.6	0.2	5.8	0.3	8.9	7.9
Aster ciliolatus	11.9	1.0	0.3	9.1	0.9	11.3	26.5
Carduus nutans	15.1	2.1	0.2	9.9	1.5	13.7	17.0
Cirsium arvense	15.9	2.3	0.3	8.8	3.4	14.8	16.5
Aster brachyactis	18.7	5.3	0.1	12.3	0.6	18.3	8.2
Echinacea angustifolia	18.0	2.8	0.1	11.2	1.3	15.4	24.2
Helianthus petiolaris	16.2	4.8	0.6	8.9	1.1	15.4	19.2
Solidago mollis	18.6	4.3	0.9	9.3	0.9	15.4	19.3
Sonchus arvensis	22.5	3.3	0.3	14.5	2.6	20.7	6.1
Tanacetum vulgare	22.8	3.2	0.7	17.1	1.6	22.6	12.4
Taraxacum officinale	24.2	4.3	0.3	10.1	7.8	22.5	37.8
Xanthium strumarium	9.4	3.8	0.5	4.3	0.4	9.0	11.0
Matricaria matricarioides	15.7	3.4	0.2	9.2	2.3	15.1	26.5
Lythrum salicaria	12.8	2.1	0.2	8.6	0.7	11.6	9.9
Polygonum amphibium	24.4	1.5	0.3	16.8	4.9	23.5	12.7
Anemone patens	14.2	1.3	0.2	9.3	0.4	11.2	7.6
Matteuccia struthiopteris	21.9	2.1	0.6	15.6	2.1	20.4	9.0
Acorus calamus	26.4	12.5	1.0	11.7	0.7	25.9	6.5

Table 2. Yield of crude methanol extract and various fractions from plant species used in the screening study, continued.

Plant Species	Crude Methanol Extract (g)	Hexane Fraction (g)	Chloroform Fraction (g)	Methanol Fraction (g)	Aqueous Fraction (g)	Recovery (g)	Boiling Water Fraction (g)
Artemisia frigida	13.8	2.9	2.1	8.3	0.5	13.8	7.6
Senecio vulgaris	18.2	3.1	0.3	8.6	3.5	15.5	9.0
Solidago missouriensis	17.3	2.8	2.0	8.4	1.6	14.8	6.5
Asparagus officinalis	28.8	0.4	4.5	12.1	9.5	26.5	9.9
Alisma plantago-aquatica	20.9	3.7	2.8	12.9	1.3	20.7	12.7
Thalictrum venulosum	12.9	1.6	0.2	9.2	0.9	11.9	6.8
Rhus radicans	18.7	3.9	0.2	13.0	0.1	17.2	1.3
Lepidium apetalum	11.1	2.8	0.2	6.4	0.3	9.7	2.4
Arthemisia absinthium	20.2	3.5	2.0	9.9	3.8	19.2	7.4
Leonurus cardiaca	11.8	2.2	0.5	6.3	0.9	9.9	4.6
Lithospermum incisum	17.8	1.3	0.3	6.2	6.7	14.5	2.9
Cladina stellaris	5.6	1.2	1.6	1.4	0.1	4.3	0.7
Lycopodium obscurum	16.3	2.7	0.8	3.8	4.2	11.5	1.3
Epilobium angustifolium	26.4	1.0	0.7	10.9	6.0	18.6	3.8
Inonutus obliquus	5.2	0.4	0.5	1.5	1.2	3.6	9.5
Aralia nudicaulis	10.8	3.0	0.2	6.4	0.4	10.0	2.7
Mentha arvensis	13.6	2.6	0.5	2.3	6.4	11.8	9.0
Equisetum sylvaticum	15.8	2.4	0.2	3.6	5.8	12.0	4.8
Melilotus alba	17.3	1.8	0.2	9.7	3.5	15.2	3.7
Rumex fennicus	26.7	1.2	0.9	12.6	8.1	22.8	3.8

Table 2. Quantity (g) and percentage (%) of fractions extracted from five plant species collected in Saskatchewan in 1999

3.2. Screening of Extracts for Anticancer Properties *in vitro*

Materials and Methods

3.2.1. Tumor Cell Lines

Three cancer cell lines are used for screening anticancer activity of the plant extracts: MDA-MB-231 ATCC (breast carcinoma), NCI-417-P33 (lung carcinoma) and WiDr ATCC (colon carcinoma). Cells were grown in RPMI 1640 medium containing 10% fetal calf serum (FCS) and antibiotics (penicillin 100 units/mL and streptomycin 100 µg/mL) and the medium was changed twice a week. The cells were grown in either 75 cm^2 or 25 cm^2 tissue culture flasks and cultured at 37°C in a humidified atmosphere of 5% CO_2 in air. Generally, the cells are kept under liquid nitrogen until use.

3.2.2. Cell Proliferation Assay (in vitro bioassays)

Anticancer activity of the plant extracts is evaluated using the CellTiter 96™ Non-Radioactive Cell Proliferation Assay as follows:

Step No.	Analytical prcedure
1.	Prepare 96 well plate with plant extract (100µg/mL)
2.	Incubate 37°C, 24-72 h, 5% CO_2
3.	Add dye solution
4.	Incubate 37°C, 4 h, 5% CO_2
5.	Add solubilization/stop solution
6.	Incubate overnight
7.	Record absorbance at 550-600 nm using ELISA plate reader

The CellTiter 96 Assay is based on the cellular conversion of a tetrazolium salt into a formazan product that is easily detected using an ELISA plate reader. The assay is performed by the addition of a premixed optimized Dye Solution to culture wells of a 96 well plate containing various concentrations of growth factor or test substance to be measured. During a 4-hour incubation, living cells reduce the soluble yellow tetrazolium

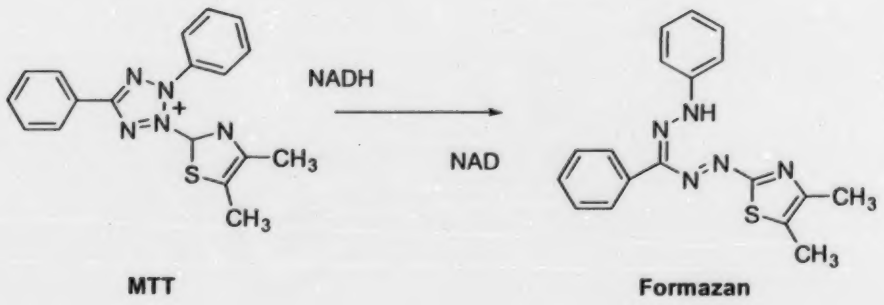


Fig. 3. Molecular structure of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and its corresponding reaction product formazan.

component of the Dye Solution (MTT) to insoluble purple formazan crystals (Fig. 7). The Solubilization/Stop Solution is then added to the culture wells to solubilize the formazan product and the absorbance at 595 nm is recorded using an ELISA reader. The intensity of the color absorbance read is directly proportional to the level of proliferation or viability (Sieuwerts, et al. 1995; Promega Technical Bulletin, Instructions for the use of products G4000 and G4100, 1992; TACS™ MTT Cell Proliferation/Viability Assay, R & D Systems).

3.2.3 Standard CellTiter™ 96 Assay Protocol for 96 Well Plates

Preparation of the Plant Extract Solutions: A solution with final concentration 2 mg/mL of each plant extract was prepared by completely dissolving 10 mg of each extract to be tested in 0.1 mL DMSO, 20 µL Tween 80 and 4.9 mL RPMI 1640 medium (without FCS and 100 µL of assay medium (without plant extract) was added into blank control wells, 50 µL into the negative control wells, 49.5 µL into the wells for the highest concentration of the positive control (rows D, E, F, columns 10, 11 and 12) and 45 µL to the remaining wells. 5.5 µL Of the positive control were added into the corresponding wells, and 5 µL of the above plant extract solutions (containing 10 µg of plant extract) was then added in to the remaining wells. Each extract is prepared in triplicate.

50 µL 2-Fold serial dilutions were performed for the positive control (Fig. 8). The plate was then equilibrated at 37°C in a humidified 5% CO_2 atmosphere while preparing the cells to be used for the assay.antibiotics).

Preparation of the Positive Control: 9.2 mg Of Pimozide were dissolved in 0.2 mL DMSO, 40 µL Tween 80 and 9.8 mL RPMI 1640 medium (without FCS and antibiotics).

Preparation of Assay Plates: According to the NCI's Developmental Therapeutics Program (DTP), natural product extracts (microorganism, marine plant, marine animal, or terrestrial plant) are prescreened at a concentration of 100 µg/ml. Thus,

Harvest Cells Used for Assay: Anchorage-dependent cell lines WiDr and MDA-MB-231 were harvested first by trypsinization and then were transferred from the culture flask into a centrifuge tube. NCI-417-P33 tumor line grows in suspension, so trypsinization is not required. After centrifugation (5.4 min at 1600 rpm), the supernatant was discarded and the pellet was resuspended in fresh culture media. An aliquot was transferred into the new culture flask with fresh medium and incubated. The dilution factor of the passaging was recorded and the number of the remaining cells was determined using a Cell Coulter Counter. A suspension to a final concentration of 1×10^5/mL in assay medium was prepared. 50µL Of the cell suspension (5,000 cells) was dispensed into all the wells of the pre-equilibrated 96 well plate, except for the blank control. The plate was incubated at 37°C for 48 h in a humidified 5% CO_2 atmosphere.

Color Development and Recording of Data:15 µL Of the Dye Solution was added to each well and the plate was incubated at 37°C for 4 h in a humidified 5% CO_2 atmosphere. After 4 h, 100 µL of the Solubilization/Stop Solution was added to each well. The plate was now allowed to stand overnight in a sealed container with a humidified atmosphere at 4°C. After this period of time the plate was incubated at 37°C for 15-20 min in a humidified 5% CO_2 atmosphere to completely solubilize the formazan crystals before recording of data. The absorbance at 595 nm wavelength was recorded using a Bio-Rad Model 3550 Microplate Reader.

Data Calculation: The growth ratio of cancer cells under different concentrations of each plant fraction was calculated by the following formula:

$Y\ (\%) = (1-T/C)\times100$

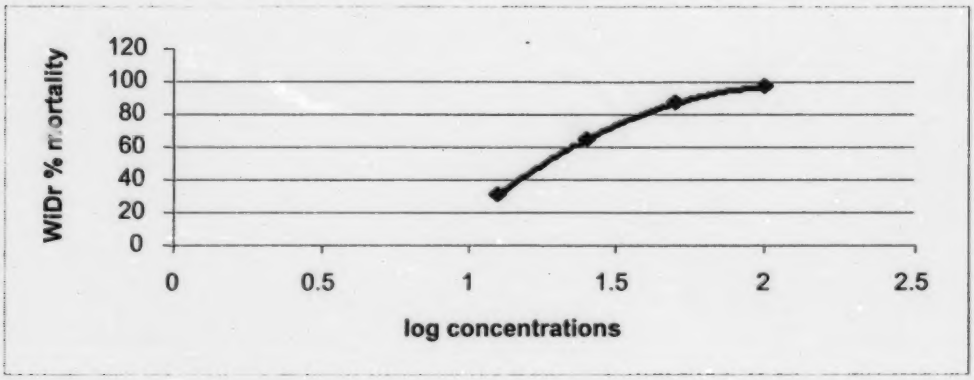
Where: Y= Growth ratio of cancer cell in each sample concentration (mortality)
T=Mean absorbance read of treated
C=Mean absorbance read of control

3.2.4. Determination of IC_{50} values

Selected plant extracts were evaluated in triplicate at concentrations of 100, 50, 25 and 12.5 µg/mL against tumor cell lines. 90 µL of assay medium was added into wells that contain the highest concentration of plant extract tested (rows A and E). Also 100 µL of medium was added into blank control (rows A and B, columns 10, 11 and 12) and

50 µL to negative control wells (rows C and D, columns 10, 11 and 12) and the remaining wells. The first concentration of 100 µg/mL was prepared by addition of 10 µL of the desired plant extract solution (see 2.3.1) into the selected wells (rows A and E). Using a multichannel pipettor, 50 µL 2-fold serial dilutions for the extracts across the well plate was performed from column to column (Fig. 9).

The plate was then equilibrated at 37°C in a humidified 5% CO_2 atmosphere while preparing the cells to be used for the assay (section 2.3.1.4). 50 µL Of the 1×10^5cells/mL suspension (5,000 cells) was dispensed into all the wells of the pre-equilibrated 96 well plate, except for the blank control. The total volume in the plate should now be 100 µL/well. The plate was incubated at 37°C for 48 h in a humidified 5% CO_2 atmosphere. Color Development and Recording of Data was explained in section 2.3.1.5 and Data Calculation in section 2.3.1.6.



The IC_{50} value is the concentration of plant extract required to kill 50% of cancer cells. It is determined from a dose response curve using four concentrations: 12.5, 25, 50 and 100 µg/mL. Analysis is done in triplicate for each concentration. The lower the IC_{50} value, the higher the cytotoxic activity of the plant extract. An example for calculating the IC_{50} value is shown in Figure 3.

Table . Percentage mortality of the most active fractions against MDA-MB-231 (Human Breast Cancer Cell Line).

No.	Plant Extract	Plant Species	Common Name	MDA-MB 231 % mortality	IC_{50} (µg/mL)
1	002 - Hex	*Lithospermum incisum*	Narrow-leaved puccoon	100.0	24.1
2	002 - $CHCl_3$	*Lithospermum incisum*	Narrow-leaved puccoon	100.0	18.7
3	075 - Hex	*Alisma plantago-aquatica*	Common water-plantain	99.9	75.3
4	047 - Hex	*Xanthium strumarium*	Cocklebur	99.1	24.7
5	075 - $CHCl_3$	*Alisma plantago-aquatica*	Common water-plantain	98.4	58.0
6	082 - Hex	*Matricaria matricarioides*	Pineappleweed	97.1	60.2
7	065 - Hex	*Sonchus arvensis*	Perennial sow-thistle	96.6	76.6
8	037 - Hex	*Solidago mollis*	Velvety goldenrod	96.6	60.0
9	064 - Hex	*Solidago missouriensis*	Low goldenrod	96.5	62.0
10	047 - $CHCl_3$	*Xanthium strumarium*	Cocklebur	95.8	6.2
11	074 - Hex	*Echinacea angustifolia*	Narrow-leaved purple coneflower	95.7	20.3
12	082 - $CHCl_3$	*Matricaria matricarioides*	Pineappleweed	95.5	44.3
13	021 - $CHCl_3$	*Artemisia ludoviciana*	Prairie sage	94.7	18.9
14	064 - $CHCl_3$	*Solidago missouriensis*	Low goldenrod	94.5	51.8
15	027 - Hex	*Cladina stellaris*	Northern reindeer lichen	94.4	57.9
16	008 - $CHCl_3$	*Artemisia frigida*	Pasture sage	93.9	18.5
17	035 - $CHCl_3$	*Carduus nutans*	Nodding thistle	93.2	40.9
18	070 - $CHCl_3$	*Tanacetum vulgare*	Tansy	92.5	5.6
19	036 - Hex	*Helianthus petiolaris*	Prairie sunflower	92.0	65.3
20	074 - $CHCl_3$	*Echinacea angustifolia*	Narrow-leaved purple coneflower	91.3	83.7
21	070 - Hex	*Tanacetum vulgare*	Tansy	91.3	40.4
22	043 - Hex	*Artemisia vulgaris*	Common wormwood	91.2	55.8
23	036 - $CHCl_3$	*Helianthus petiolaris*	Prairie sunflower	90.8	23.0
24	009 - Hex	*Artemisia campestris*	Plains wormwood	89.7	11.4
25	065 - $CHCl_3$	*Sonchus arvensis*	Perennial sow-thistle	86.4	55.4
26	033 - MeOH	*Cirsium arvense*	Canada thistle	86.1	60.5
27	072 - Hex	*Aster brachyactis*	Rayless aster	85.6	49.6
28	021 - Hex	*Artemisia ludoviciana*	Prairie sage	85.5	51.8
29	072 - B.w.	*Aster brachyactis*	Rayless aster	85.5	11.4
30	043 - $CHCl_3$	*Artemisia vulgaris*	Common wormwood	85.0	80.3
31	003 - $CHCl_3$	*Aralia nudicaulis*	Wild sarsaparilla	84.9	44.5
32	069 - $CHCl_3$	*Asparagus officinalis*	Asparagus	84.8	17.2
33	065 - MeOH	*Sonchus arvensis*	Perennial sow-thistle	84.3	91.2
34	070 - W	*Tanacetum vulgare*	Tansy	81.9	69.1
35	064 - W	*Solidago missouriensis*	Low goldenrod	81.5	60.3
36	043 - B.w.	*Artemisia vulgaris*	Common wormwood	80.2	>100
37	023 - $CHCl_3$	*Senecio vulgaris*	Common groundsel	79.9	81.2
38	037 - $CHCl_3$	*Solidago mollis*	Velvety goldenrod	78.6	35.2
39	071 - B.w.	*Lythrum salicaria*	Purple loosestrife	78.1	60.2
40	001 - $CHCl_3$	*Anemone patens*	Crocus anemone	77.8	71.0
41	021 - B.w.	*Artemisia ludoviciana*	Prairie sage	76.7	>100
42	047 - MeOH	*Xanthium strumarium*	Cocklebur	75.5	51.6
43	009 - $CHCl_3$	*Artemisia campestris*	Plains wormwood	74.4	80.0
44	024 - W	*Mentha arvensis*	Wild mint	73.9	78.5
45	071 - W	*Lythrum salicaria*	Purple loosestrife	71.0	58.9
46	024 - MeOH	*Mentha arvensis*	Wild mint	69.8	70.3
47	071 - MeOH	*Lythrum salicaria*	Purple loosestrife	66.6	59.5

Table . Percentage mortality of the most active fractions against MDA-MB-231 (Human Breast Cancer Cell Line) continued.

No.	Plant Extract	Plant Species	Common Name	MDA-MB 231 % mortality	IC_{50} (µg/mL)
48	003 - Hex	*Aralia nudicaulis*	Wild sarsaparilla	66.5	88.3
49	025 - $CHCl_3$	*Equisetum sylvaticum*	Woodland horsetail	63.3	92.2
50	008 - Hex	*Artemisia frigida*	Pasture sage	62.9	91.4
51	029 - $CHCl_3$	*Melilotus alba*	White sweet-clover	59.7	78.9
52	071 - $CHCl_3$	*Lythrum salicaria*	Purple loosestrife	57.9	93.5
53	004 - $CHCl_3$	*Thalictrum venulosum*	Veiny meadow-rue	55.5	>100
54	083 - Hex	*Inonutus obliquus*	Chaga, Birch Mushroom	55.4	19.1
55	025 - W	*Equisetum sylvaticum*	Woodland horsetail	55.0	78.8
56	071 - Hex	*Lythrum salicaria*	Purple loosestrife	53.0	90.8
57	001 - Hex	*Anemone patens*	Crocus anemone	42.2	83.5
58	005 - $CHCl_3$	*Rhus radicans*	Poison-ivy	39.2	32.5
59	076 - W	*Polygonum amphibium*	Water smartweed	36.9	67.8

Hex = Hexane fraction MeOH = Methanol fraction B.w. = Boiling water fraction
$CHCl_3$ = Chloroform fraction W = Aqueous fraction

Table . Percentage mortality of the most active fractions against WiDr (Human Colon Cancer Cell line).

No.	Plant Extract	Plant Species	Common Name	WiDr % mortality	IC_{50} (µg/mL)
1	002 - Hex	*Lithospermum incisum*	Narrow-leaved puccoon	100.0	9.4
2	002 - $CHCl_3$	*Lithospermum incisum*	Narrow-leaved puccoon	100.0	10.9
3	074 - Hex	*Echinacea angustifolia*	Narrow-leaved purple coneflower	100.0	17.7
4	070 - Hex	*Tanacetum vulgare*	Tansy	100.0	15.2
5	027 - Hex	*Cladina stellaris*	Northern reindeer lichen	99.6	23.3
6	075 - $CHCl_3$	*Alisma plantago-aquatica*	Common water-plantain	99.5	25.6
7	047 - Hex	*Xanthium strumarium*	Cocklebur	99.1	12.2
8	070 - $CHCl_3$	*Tanacetum vulgare*	Tansy	98.9	9.1
9	064 - Hex	*Solidago missouriensis*	Low goldenrod	98.3	53.4
10	075 - Hex	*Alisma plantago-aquatica*	Common water-plantain	97.8	51.4
11	008 - $CHCl_3$	*Artemisia frigida*	Pasture sage	96.9	20.6
12	047 - $CHCl_3$	*Xanthium strumarium*	Cocklebur	95.7	3.3
13	037 - Hex	*Solidago mollis*	Velvety goldenrod	95.6	41.9
14	082 - Hex	*Matricaria matricarioides*	Pineappleweed	95.5	29.9
15	027 - $CHCl_3$	*Cladina stellaris*	Northern reindeer lichen	94.7	17.3
16	036 - Hex	*Helianthus petiolaris*	Prairie sunflower	94.7	67.5
17	033 - Hex	*Cirsium arvense*	Canada thistle	94.4	50.5
18	064 - $CHCl_3$	*Solidago missouriensis*	Low goldenrod	94.3	54.2
19	065 - $CHCl_3$	*Sonchus arvensis*	Perennial sow-thistle	93.5	28.2
20	082 - $CHCl_3$	*Matricaria matricarioides*	Pineappleweed	93.3	25.9
21	021 - $CHCl_3$	*Artemisia ludoviciana*	Prairie sage	93.1	18.1
22	009 - Hex	*Artemisia campestris*	Plains wormwood	92.8	19.4
23	035 - Hex	*Carduus nutans*	Nodding thistle	92.1	44.9
24	069 - $CHCl_3$	*Asparagus officinalis*	Asparagus	91.4	9.7
25	004 - $CHCl_3$	*Thalictrum venulosum*	Veiny meadow-rue	90.5	13.7
26	035 - $CHCl_3$	*Carduus nutans*	Nodding thistle	90.4	28.7
27	074 - $CHCl_3$	*Echinacea angustifolia*	Narrow-leaved purple coneflower	88.7	33.7
28	064 - W	*Solidago missouriensis*	Low goldenrod	88.3	45.7
29	021 - Hex	*Artemisia ludoviciana*	Prairie sage	87.8	63.0
30	075 - B.w.	*Alisma plantago-aquatica*	Common water-plantain	87.4	>100
31	036 - $CHCl_3$	*Helianthus petiolaris*	Prairie sunflower	87.2	23.2
32	071 - B.w.	*Lythrum salicaria*	Purple loosestrife	87.2	79.1
33	016 - $CHCl_3$	*Artemisia absinthium*	Absinthe	86.8	48.4
34	001 - $CHCl_3$	*Anemone patens*	Crocus anemone	86.8	2.9
35	083 - Hex	*Inonutus obliquus*	Chaga, Birch Mushroom	86.7	>100
36	033 - MeOH	*Cirsium arvense*	Canada thistle	85.8	34.2
37	029 - $CHCl_3$	*Melilotus alba*	White sweet-clover	85.2	77.4
38	033 - $CHCl_3$	*Cirsium arvense*	Canada thistle	84.9	51.3
39	003 - $CHCl_3$	*Aralia nudicaulis*	Wild sarsaparilla	84.5	67.4
40	024 - W	*Mentha arvensis*	Wild mint	83.8	>100
41	001 - Hex	*Anemone patens*	Crocus anemone	83.7	26.5
42	071 - W	*Lythrum salicaria*	Purple loosestrife	83.2	74.1
43	058 - Hex	*Acorus calamus*	Sweet flag	83.2	83.7
44	065 - MeOH	*Sonchus arvensis*	Perennial sow-thistle	82.8	19.4
45	069 - Hex	*Asparagus officinalis*	Asparagus	82.4	>100
46	043 - $CHCl_3$	*Artemisia vulgaris*	Common wormwood	82.1	32.2
47	071 - MeOH	*Lythrum salicaria*	Purple loosestrife	82.1	67.4

Table . Percentage mortality of the most active fractions against WiDr (Human Colon Cancer Cell line) continued.

No.	Plant Extract	Plant Species	Common Name	WiDr % mortality	IC_{50} (µg/mL)
48	042 - Hex	*Leonurus cardiaca*	Motherwort	81.8	-
49	061 - MeOH	*Epilobium angustifolium*	Fireweed	81.2	>100
50	071 - Hex	*Lythrum salicaria*	Purple loosestrife	81.1	>100
51	071 - $CHCl_3$	*Lythrum salicaria*	Purple loosestrife	80.2	74.7
52	023 - $CHCl_3$	*Senecio vulgaris*	Common groundsel	80.2	>100
53	016 - Hex	*Artemisia absinthium*	Absinthe	79.6	42.1
54	059 - $CHCl_3$	*Aster ciliolatus*	Lindley's aster	79.3	24.2
55	059 - B.w.	*Aster ciliolatus*	Lindley's aster	78.0	70.4
56	003 - Hex	*Aralia nudicaulis*	Wild sarsaparilla	77.4	71.3
57	025 - $CHCl_3$	*Equisetum sylvaticum*	Woodland horsetail	77.3	69.2
58	059 - Hex	*Aster ciliolatus*	Lindley's aster	77.2	18.6
59	008 - Hex	*Artemisia frigida*	Pasture sage	76.9	93.7
60	024 - MeOH	*Mentha arvensis*	Wild mint	76.5	80.7
61	042 - $CHCl_3$	*Leonurus cardiaca*	Motherwort	76.3	41.2
62	025 - Hex	*Equisetum sylvaticum*	Woodland horsetail	76.1	35.1
63	061 - Hex	*Epilobium angustifolium*	Fireweed	75.6	55.7
64	037 - $CHCl_3$	*Solidago mollis*	Velvety goldenrod	75.2	27.3
65	004 - Hex	*Thalictrum venulosum*	Veiny meadow-rue	75.2	39.3
66	083 - $CHCl_3$	*Inonutus obliquus*	Chaga, Birch Mushroom	74.8	79.7
67	009 - $CHCl_3$	*Artemisia campestris*	Plains wormwood	74.3	47.2
68	010 - $CHCl_3$	*Lepidium apetalum*	Common pepper-grass	74.2	15.1
69	072 - Hex	*Aster brachyactis*	Rayless aster	70.0	43.2
70	005 - $CHCl_3$	*Rhus radicans*	Poison-ivy	69.3	30.6
71	076 - W	*Polygonum amphibium*	Water smartweed	66.1	68.4
72	072 - $CHCl_3$	*Aster brachyactis*	Rayless aster	65.4	29.2
73	065 - Hex	*Sonchus arvensis*	Perennial sow-thistle	64.4	15.1
74	023 - Hex	*Senecio vulgaris*	Common groundsel	63.1	94.7

Hex = Hexane fraction MeOH = Methanol fraction B.w. = Boiling water fraction
$CHCl_3$ = Chloroform fraction W = Aqueous fraction

Table . Percentage mortality of the most active fractions against NCI-417 (Human Lung Cancer Cell line)

No.	Plant Extract	Plant Species	Common Name	NCI-417 % mortality	IC_{50} (µg/mL)
1	002 - $CHCl_3$	*Lithospermum incisum*	Narrow-leaved puccoon	100.0	5.3
2	002 - Hex	*Lithospermum incisum*	Narrow-leaved puccoon	100.0	10.6
3	016 - Hex	*Artemisia absinthium*	Absinthe	100.0	44.6
4	075 - Hex	*Alisma plantago-aquatica*	Common water-plantain	100.0	59.7
5	047 - Hex	*Xanthium strumarium*	Cocklebur	99.2	14.2
6	016 - $CHCl_3$	*Artemisia absinthium*	Absinthe	99.2	69.5
7	075 - $CHCl_3$	*Alisma plantago-aquatica*	Common water-plantain	99.0	38.9
8	027 - Hex	*Cladina stellaris*	Northern reindeer lichen	98.1	14.6
9	082 - Hex	*Matricaria matricarioides*	Pineappleweed	96.8	49.6
10	074 - Hex	*Echinacea angustifolia*	Narrow-leaved purple coneflower	96.0	4.0
11	064 - Hex	*Solidago missouriensis*	Low goldenrod	95.2	53.1
12	047 - $CHCl_3$	*Xanthium strumarium*	Cocklebur	95.0	0.02
13	027 - $CHCl_3$	*Cladina stellaris*	Northern reindeer lichen	95.0	16.3
14	037 - Hex	*Solidago mollis*	Velvety goldenrod	94.9	36.4
15	033 - Hex	*Cirsium arvense*	Canada thistle	94.6	51.6
16	069 - Hex	*Asparagus officinalis*	Asparagus	94.5	77.3
17	021 - $CHCl_3$	*Artemisia ludoviciana*	Prairie sage	94.3	11.8
18	082 - $CHCl_3$	*Matricaria matricarioides*	Pineappleweed	94.1	88.8
19	029 - $CHCl_3$	*Melilotus alba*	White sweet-clover	94.0	68.3
20	035 - Hex	*Carduus nutans*	Nodding thistle	93.5	38.0
21	065 - Hex	*Sonchus arvensis*	Perennial sow-thistle	93.3	80.2
22	003 - $CHCl_3$	*Aralia nudicaulis*	Wild sarsaparilla	93.1	36.7
23	064 - $CHCl_3$	*Solidago missouriensis*	Low goldenrod	93.1	55.7
24	070 - $CHCl_3$	*Tanacetum vulgare*	Tansy	92.8	2.4
25	037 - $CHCl_3$	*Solidago mollis*	Velvety goldenrod	92.7	25.3
26	035 - $CHCl_3$	*Carduus nutans*	Nodding thistle	92.4	9.3
27	036 - $CHCl_3$	*Helianthus petiolaris*	Prairie sunflower	91.9	17.5
28	027- MeOH	*Cladina stellaris*	Northern reindeer lichen	91.7	62.2
29	075- MeOH	*Alisma plantago-aquatica*	Common water-plantain	90.9	>100
30	043 - Hex	*Artemisia vulgaris*	Common wormwood	90.9	>100
31	008 - $CHCl_3$	*Artemisia frigida*	Pasture sage	90.4	16.4
32	070 - Hex	*Tanacetum vulgare*	Tansy	90.4	30.0
33	036 - Hex	*Helianthus petiolaris*	Prairie sunflower	90.3	69.4
34	058 - Hex	*Acorus calamus*	Sweet flag	89.3	63.2
35	009 - Hex	*Artemisia campestris*	Plains wormwood	88.9	17.6
36	033 - W	*Cirsium arvense*	Canada thistle	88.7	>100
37	059 - B.w.	*Aster ciliolatus*	Lindley's aster	88.6	>100
38	043 - $CHCl_3$	*Artemisia vulgaris*	Common wormwood	88.4	48.5
39	021 - Hex	*Artemisia ludoviciana*	Prairie sage	88.0	>100
40	059 - Hex	*Aster ciliolatus*	Lindley's aster	87.6	61.5
41	074 - $CHCl_3$	*Echinacea angustifolia*	Narrow-leaved purple coneflower	85.7	38.0
42	033 - $CHCl_3$	*Cirsium arvense*	Canada thistle	85.6	85.6
43	017 - $CHCl_3$	*Taraxacum officinale*	Tansy	85.6	>100
44	035 -MeOH	*Carduus nutans*	Nodding thistle	85.2	96.7
45	082 -MeOH	*Matricaria matricarioides*	Pineappleweed	85.2	>100
46	082 - B.w.	*Matricaria matricarioides*	Pineappleweed	84.9	>100

Table . Percentage mortality of the most active fractions against NCI-417 (Human Lung Cancer Cell line) continued.

No.	Plant Extract	Plant Species	Common Name	NCI-417 % mortality	IC_{50} (µg/mL)
47	004 - $CHCl_3$	*Thalictrum venulosum*	Veiny meadow-rue	84.5	37.8
48	059 - $CHCl_3$	*Aster ciliolatus*	Lindley's aster	84.2	29.9
49	047 - B.w.	*Xanthium strumarium*	Cocklebur	83.7	57.2
50	072 - Hex	*Aster brachyactis*	Rayless aster	83.6	88.7
51	083 - Hex	*Inonutus obliquus*	Chaga, Birch Mushroom	83.3	13.2
52	009 - $CHCl_3$	*Artemisia campestris*	Plains wormwood	82.9	86.4
53	033 - B.w.	*Cirsium arvense*	Canada thistle	82.5	>100
54	065- MeOH	*Sonchus arvensis*	Perennial sow-thistle	81.0	30.8
55	033 -MeOH	*Cirsium arvense*	Canada thistle	80.5	22.0
56	003 - Hex	*Aralia nudicaulis*	Wild sarsaparilla	77.4	51.4
57	069 - $CHCl_3$	*Asparagus officinalis*	Asparagus	76.6	16.2
58	071 - $CHCl_3$	*Lythrum salicaria*	Purple loosestrife	74.0	65.1
59	071 – B.w.	*Lythrum salicaria*	Purple loosestrife	71.8	73.1
60	061 - Hex	*Epilobium angustifolium*	Fireweed	71.3	17.0
61	061 - $CHCl_3$	*Epilobium angustifolium*	Fireweed	70.3	63.6
62	025 - $CHCl_3$	*Equisetum sylvaticum*	Woodland horsetail	69.5	82.6
63	083 - $CHCl_3$	*Inonutus obliquus*	Chaga, Birch Mushroom	69.0	69.7
64	024 - Hex	*Mentha arvensis*	Wild mint	68.7	32.5
65	010 - $CHCl_3$	*Lepidium apetalum*	Common pepper-grass	67.7	77.5
66	024 - W	*Mentha arvensis*	Wild mint	65.2	77.7
67	064 - W	*Solidago missouriensis*	Low goldenrod	64.3	48.1
68	042 - $CHCl_3$	*Leonurus cardiaca*	Motherwort	63.0	77.8
69	024 - $CHCl_3$	*Mentha arvensis*	Wild mint	60.4	25.8
70	071 -MeOH	*Lythrum salicaria*	Purple loosestrife	58.4	78.5
71	024 -MeOH	*Mentha arvensis*	Wild mint	53.9	70.7
72	071 - W	*Lythrum salicaria*	Purple loosestrife	50.4	67.9
73	004 - Hex	*Thalictrum venulosum*	Veiny meadow-rue	49.8	75.3
74	005 - $CHCl_3$	*Rhus radicans*	Poison-ivy	31.3	86.4
75	001 - $CHCl_3$	*Anemone patens*	Crocus anemone	-	11.2
76	001 - Hex	*Anemone patens*	Crocus anemone	-	49.9
77	071 - Hex	*Lythrum salicaria*	Purple loosestrife	-	75.3

Hex = Hexane fraction MeOH = Methanol fraction B.w. = Boiling water fraction
$CHCl_3$ = Chloroform fraction W = Aqueous fraction

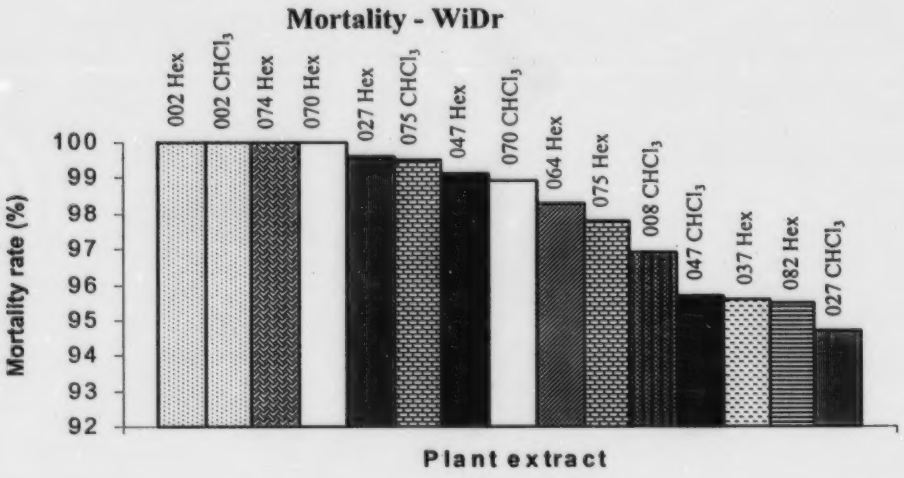


Figure xx. Mortality rate of human colon cancer cell line WiDr treated with plant extracts. The bars represent the mean mortality values for triplicate readings; the mortality rate of control (no treatment) is 0.

002 *Lithospermum incisum*
074 *Echinacea angustifolia*
070 *Tanacetum vulgare*
027 *Cladina stellaris*
075 *Alisma plantago-aquatica*
047 *Xanthium strumarium*
064 *Solidago missouriensis*
008 *Artemisia frigida*
037 *Solidago mollis*
082 *Matricaria matricarioides*

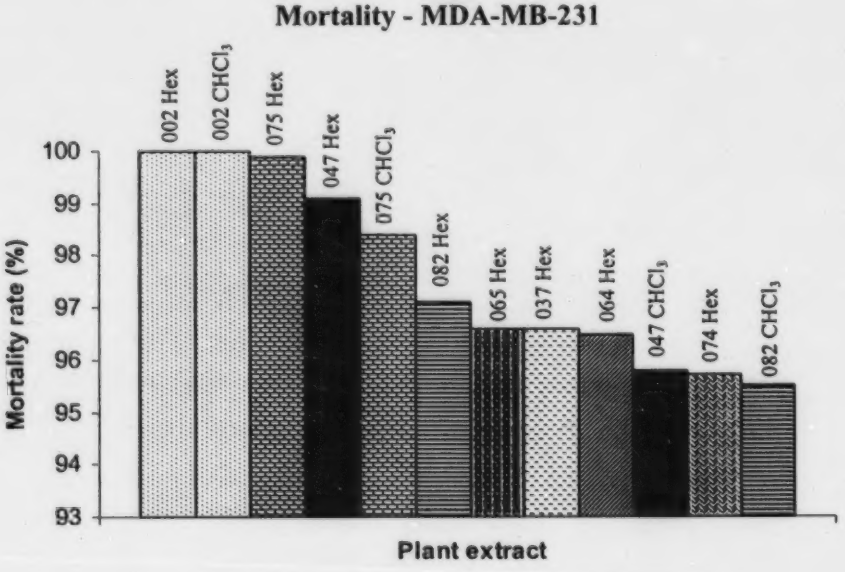


Figure xx. Mortality rate of human breast cancer cell line MDA-MB-231 treated with plant extracts. The bars represent the mean mortality values for triplicate readings; the mortality rate of control (no treatment) is 0.

002 *Lithospermum incisum*
075 *Alisma plantago-aquatica*
047 *Xanthium strumarium*
082 *Matricaria matricarioides*
065 *Sonchus arvensis*
037 *Solidago mollis*
064 *Solidago missouriensis*
074 *Echinacea angustifolia*

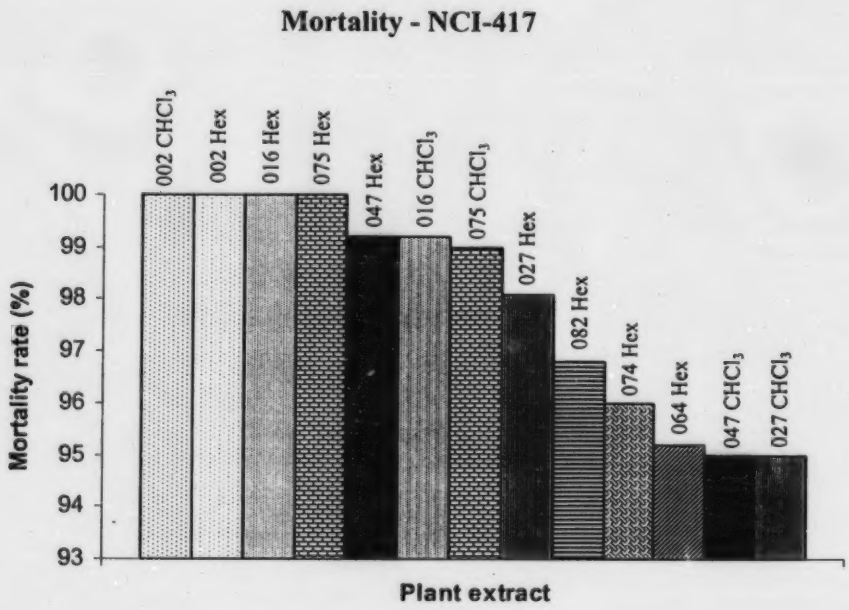


Figure xx. Mortality rate of human lung cancer cell line NCI-417 treated with plant extracts. The bars represent the mean mortality values of triplicate reading; the mortality rate of control (no treatment) is 0.

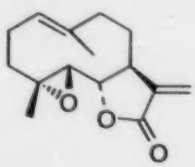
002 *Lithospermum incisum*
016 *Arthemisia absinthium*
075 *Alisma plantago-aquatica*
047 *Xanthium strumarium*
027 *Cladina stellaris*
082 *Matricaria matricarioides*
074 *Echinacea angustifolia*
064 *Solidago missouriensis*

Table 13. Cytotoxic activity of plant parts of tansy (*Tanacetum vulgare*) expressed as percentage mortality of three human cancer cell lines (colon, breast and lung).

No.	Tansy Plant Part Extract	WiDr (colon) % mortality	MDA-MB-231(breast) % mortality	NCI-417 (lung) % mortality
1.	Leaves - $CHCl_3$	97.1	93.2	93.9
2.	Flowers - Hexane	95.6	60.8	92.4
3.	Leaves - Hexane	94.8	47.7	85.0
4.	Flowers - $CHCl_3$	93.4	85.2	82.6
5.	Stems - Hexane	91.8	65.9	88.1
6.	Stems - $CHCl_3$	90.0	52.6	86.4

Whereas no report has been found on cytotoxic activity of *T. vulgare*, low concentration of sesquiterpene lactone parthenolide (1), a clinically useful agent and migraine prophylaxis principle, from the closely related plant species *T. parthenium* (Feverfew),

were reported to inhibit *in vitro* growth of two tumor cell lines (mouse fibrosarcoma MN-11 and human lymphoma TK6) in a cytostatic fashion (Ross, et al. 1999).



Parthenolide (1)

4. Fractionation of Promising Extracts using Bioassay-guided Fractionation Strategy

Thin Layer Chromatography (TLC)

Analytical TLC was performed on aluminum - backed plates of silica gel 60 F_{254} (layer thickness 0.2 mm, 20 x 20 cm; E. Merck, Darmstadt, Germany). The developing solvent system for the $CHCl_3$ extract of the fruits of cocklebur was Hex-EtOAc (2:3). The developed plates were air-dried, observed under UV_{254} light, and then immersed in Phosphomolybdic acid reagent (staining solution). Metabolites were visualized after color development upon heating.

Fractionation by flash column chromatography (FCC) using Hex-EtOAc system and gradient elution

The $CHCl_3$ extract of the fruits of cocklebur was subjected to fractionation by flash column chromatography (FCC). The column (60 cm in length, 25 mm i.d.) was packed with a slurry of silica gel (particle size 230-400 mesh, EM Science) in Hex-EtOAc (2:1). The dried $CHCl_3$ extract (about 0.2 g) was dissolved in a minimal amount (about 1 mL) of the solvent system and applied with a pipette to the top of the silica. The column was subsequently eluted with a solvent gradient of Hex-EtOAc (2:1, 1:1, 2:3, EtOAc 100%). A total of 112 fractions were collected, 7 mL each fraction. Fractions 1-33 were collected with the first solvent gradient, fractions 34-72 with the second, fractions 73-100 with the

third and fractions 101-112 with the fourth solvent gradient. All fractions eluted were analysed by TLC.

High Performance Liquid Chromatography(HPLC) analysis

Analytical HPLC was performed on a system consisting of a Waters Nova-Pak® C18 (150 x 3.9 mm i.d., 60 Å, 4 μm) column, and a Waters 996 Photodiode Array Detector (PDA). The injection volume was 5 μL and fractions were eluted using a solvent system with gradient (80% H_2O:20% MeCN to 40% H_2O:60% MeCN over 35 min. PDA was set to scan in the region of 192 to 400 nm and the column effluent was monitored at 315 and 210 nm. Data were collected and analyzed using Millennium[32] chromatography software (Waters Corporation, USA).

Bioassay Guided Fractionation of *Echinacea angustifolia*

Bioassay guided fractionation was performed on the following three plants species: *Echinacea angustifolia*, *Asparagus officinalis* and *Artemisia absinthium*. Results are presented here for the extraction, partitioning and fractionation of the plant species. Cytotoxic activity was observed in several of the extracts. The hexane extract from *E. angustifolia* showed high (90-97 %) mortality against all three cancer cell lines. Further fractionation of the hexane extract from *E. angustifolia* was conducted (since Feb. 19, 2001) and the results of the bioassay are discussed.

Extraction and Partitioning

The *E. angustifolia* root material used for this project was selected based on high alkylamide content from the AFIF *E. angustifolia* collection. The total amount of ground root material used for extraction was 1000 g.

E. angustifolia ground root material (1000 g) was divided into two batches and extracted with methanol (5:1) for 24 hours (3×). The crude MeOH ex. (133.4 g) was divided into two batches dissolved in water (250 ml) and further partitioned with hexane (7×, 2:1, 1:1) and $CHCl_3$ (4×; 2:1, 1:1). The extracts were combined and concentrated to

dryness yielding hexane and $CHCl_3$ extracts for batch 1 and 2. A percent mortality bioassay was performed on the crude MeOH ex., Hexane and $CHCl_3$ extracts.

Hexane Soxhlet extraction of ground root material (80 g) from *E. angustifolia* was performed by D. Dunlop as similarly described by Bauer *et al* (1989). The *E. angustifolia* samples were from the AFIF collection (30 g samples from each of # 40, 41, 51 and 75). The total ground material (120 g) was combined and mixed. Two batches (40 g each with 40 g remain) were extracted with hexane (350 ml) by Soxhlet extraction for 6 hours. The hexane extracts were pooled and concentrated to dryness. The hexane extract was analyzed by HPLC and the cytotoxic activity was screened against the three cancer cell lines as previous.

Reversed phase fractionation of hexane extract from Echinacea angustifolia (extract added as a dry powder adsorbed to reversed phase silica gel)

The hexane extract from *E. angustifolia* was further fractionated by reversed phase C18 chromatography by adding the extract to the column as a dry powder adsorbed to reversed phase silica gel as described in the experimental. Initial work was conducted by fractionating, fraction 2-36-1/CN obtained from fractionation #1 (see section 2.1.2). Fraction 2-36-1/CN (136.3 mg) was added to the column as a dry powder and eluted with a gradient of water-acetonitrile (80:20 to 30:70) (see 39-1/CN of record book). The fractions obtained from reversed phase chromatography were analyzed by HPLC (photodiode array detector). Fractions have similar composition were combined and further analyzed by HPLC. The yields of the fractions were low; therefore, the fractionation was scaled up following the same protocol. Several prominent peaks were observed in the HPLC chromatograms of the extracts with retention times (ca. minutes) of 7.0, 9.0, 10.5, 11.7, 15.6, 18.5. Further fractionation attempted to obtain purified samples of these metabolites. A scaled-up fractionation of the hexane extract (592.2 mg) of *E. angustifolia* was performed by reversed phase chromatography as described in the experimental. The extract was eluted using a water-acetonitrile gradient and the fractions collected were analyzed by HPLC (photodiode array detector). Fractions having similar

composition were combined, further analyzed by HPLC and their cytotoxic activity was screened (% mortality bioassay) against the three cancer cell lines as before. HPLC analysis of the fractions indicated the presence of metabolites as previously detected (see retention times of these metabolites listed above). There were several of the samples that showed high (> 90 % mortality) cytotoxic activity against the cancer cell lines (Table 2.4). Samples 6-61-1/CN and 10-61-1/CN were active against all three cancer cell lines.

Results

The percent crude MeOH ex. of *E. angustifolia* that was previously attained was 18.0 % from 100 g of ground root material and the % recovery (based on crude MeOH ex.) of the hexane and $CHCl_3$ extracts were 15.4 and 0.37 respectively. The percent of crude MeOH extract obtained from the present work was 13.3 % based on the total ground root material (1000 g) (Table 2.1). The lower recovery could be attributed to the larger scale of the extraction. For future experiments increased shaking could improve the methanol extraction. In this experiment the bottles were only briefly shaken periodically (4-5 times each day). The percent recoveries (based on crude MeOH ex.) of the hexane and $CHCl_3$ extracts were 9.7 and 7.3 respectively (Table 2.1). Although, the individual percent recoveries differ from the previous work the total recovery of hexane and $CHCl_3$ extracts are similar. This difference could be attributed to the partitioning (i.e. emulsions) and possibly differences in plant material source.

HPLC profile of crude chloroform extract of *X. strumarium* showing peaks of interest is presented in Fig. 3. Purification of this extract led to the isolation of four fractions whose cytotoxic activity was evaluated against the three cancer cell lines. Mortality rates above 90% were obtained for all three cell lines. Preliminary ^{1}H- and ^{13}C-NMR studies suggested compound with Rt 18.75 min to be a sesquiterpene lactone. Further structural characterization of compounds is underway.

Table 2.1 The yield and percent recovery of extracts from roots of *E. angustifolia*

Type of Extract	Yield from 1Kg of Root Material (g)	Recovery Based on Root Material (%)	Recovery Based on Crude Methanol Extract (%)
Crude MeOH	133.4	13.3	-
Hexane Extract	12.9	1.30	9.7
Chloroform Extract	9.8	0.98	7.3

1 The hexane extract (Soxhlet) was obtained from D. Dunlop. Hexane Soxhlet extraction of 80 g total *E. angustifolia*. The percent recovery was based on 80 g of ground root material : 890.5 mg; 1.1%) yield.

Table 2.2 Mortality (%) of cancer cell lines WiDR, MDA-MB-231 and NCI-417 against plant extracts from *E. angustifolia*

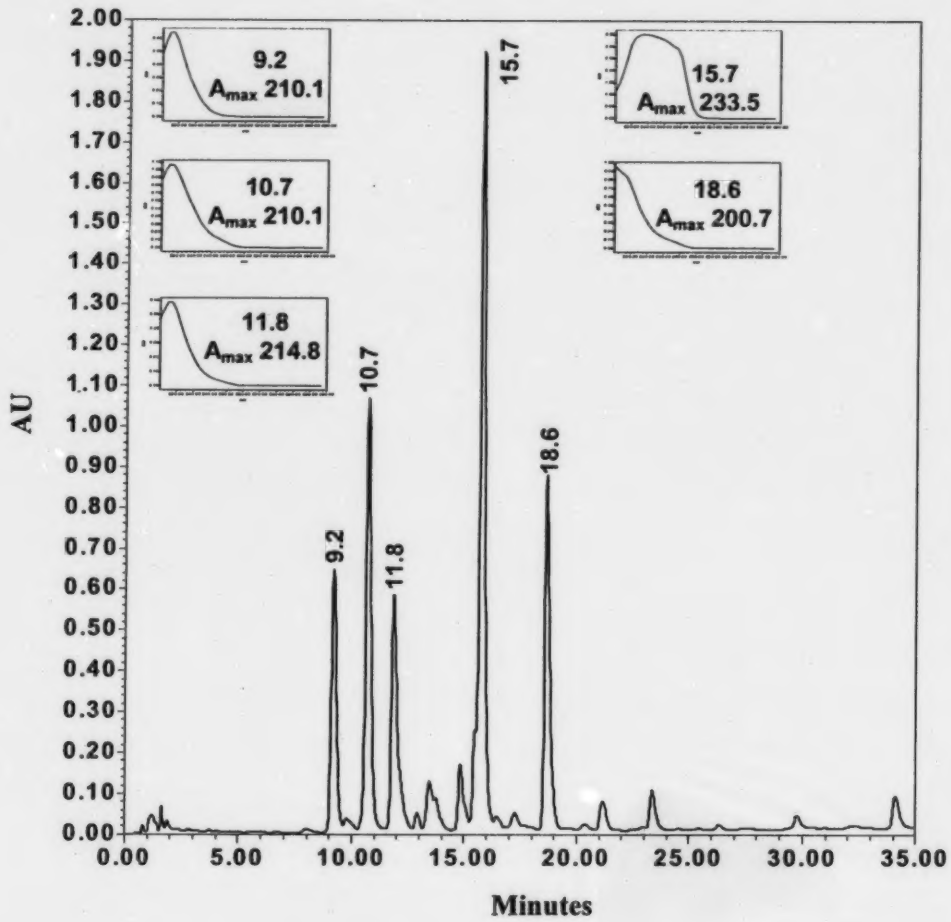
Echinacea angustifolia Root Extract	Mortality of Cancer Cells (%)		
	WiDR	MDA-MB-231	NCI-417
Crude Methanol	62.9	0	0
Hexane	96.8	89.8	92.4
Hexane (Soxhlet)	95.7	83.5	97.8
Chloroform	94.3	30.2	31.6

Table 2.4 Percent mortality of cancer cell lines against fractions from hexane extract of *E. angustifolia* root

Sample	Peak R_t (min)[1]	Amount (mg)	Mortality of Cancer Cells (%) WiDR	MDA-MB-231	NCI-417
3-61-1/CN	9.2	9.0	83.7	15.6	0.0
4-61-1/CN	9.2, 10.5	18.9	20.0	0.0	0.0
5-61-1/CN	10.5, 11.7	38.5	78.8	14.8	0.0
6-61-1/CN	**complex mixture**	**13.8**	**98.9**	**97.6**	**97.7**
7-61-1/CN	15.6	24.3	89.3	42.7	88.9
8-61-1/CN	15.6, 18.5	36.2	92.7	70.8	67.8
10-61-1/CN	**18.5**	**15.7**	**98.9**	**97.8**	**93.5**
11-61-1/CN	18.5, 11.0	12.2	**97.1**	79.1	92.0
12-61-1/CN	?	15.0	62.5	45.5	67.8

[1] The prominent peak in each sample is indicated. The metabolites are identified by their peak retention time (minutes) from the HPLC chromatograms. Note: No UV-adsorbing peaks were detected in sample 12-61-1/CN.

Echinacea angustifolia extract



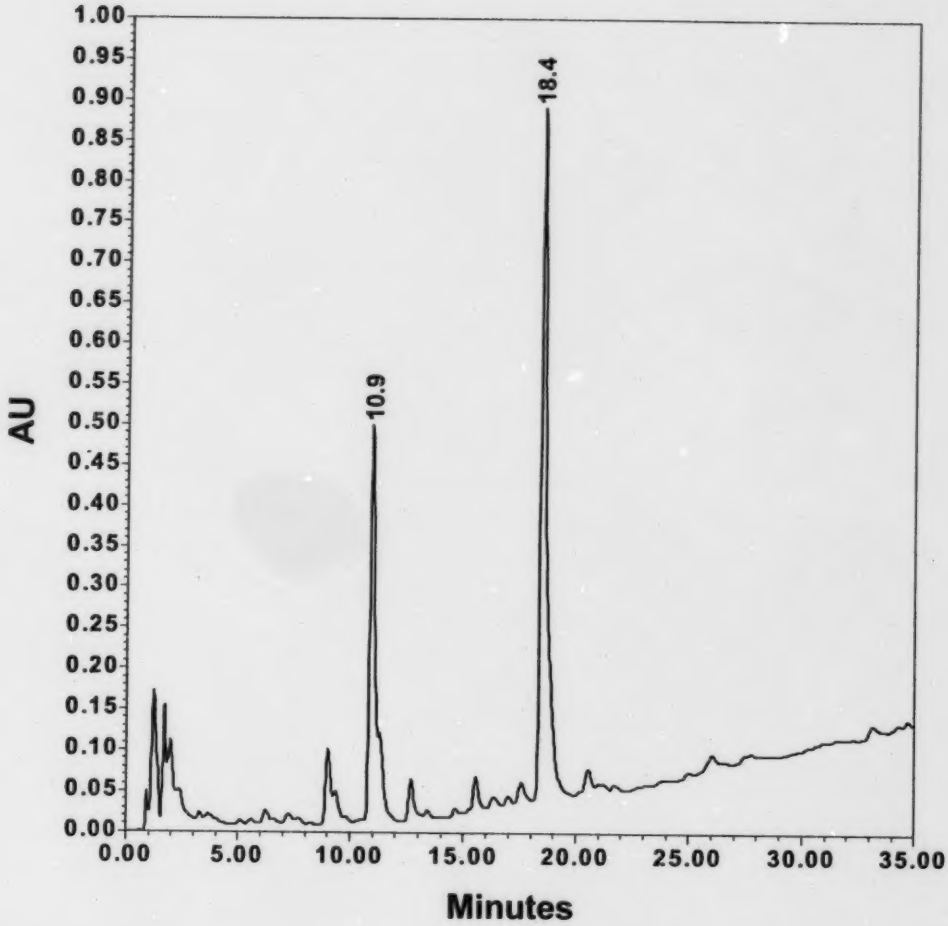

AU
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
10.9
18.4
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
Minutes

Fig. 3. HPLC chromatogram of the chloroform extract of the *Xanthium strumarium* fruits at 210 nm.

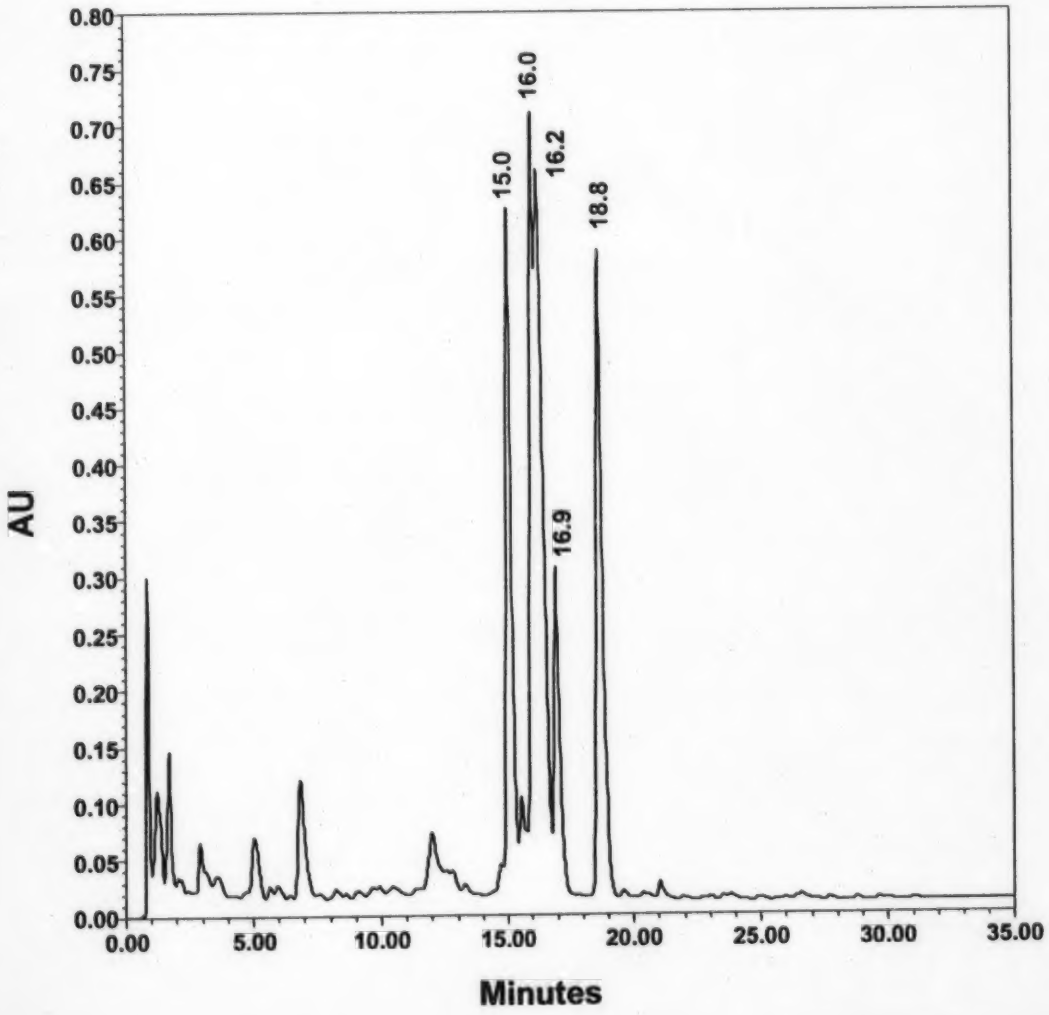
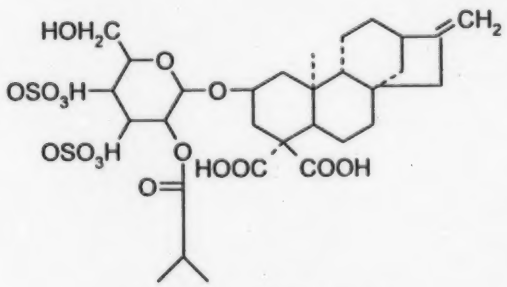


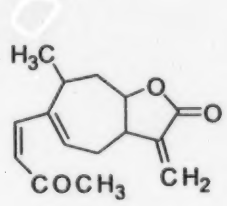
Fig. 3. HPLC chromatogram of the chloroform extract of the *Xanthium strumarium* fruits at 210 nm.

Cocklebur (*Xanthium strumarium*) is found throughout the United States from the south of Canada to Mexico and is a common weed found in corn and soybean fields. Common cocklebur complexes have developed in ecosystems disturbed by man on every continent. (McMillan, C. 1975a).

It has been reported that xanthatin (**3**), an antimicrobial agent from *X. strumarium*, exhibited cytotoxic actvity against two murine lymphocytic leukemia cell- lines P-388 and L-1210 and against the human origin-bronchial epidermoid carcinoma NSCLC-N6 (Non-Small Cell Lung Cancer), a very resrstant cell line (Roussakis, 1994).



Carboxyatractyloside (**2**)



Xanthatin (**3**)

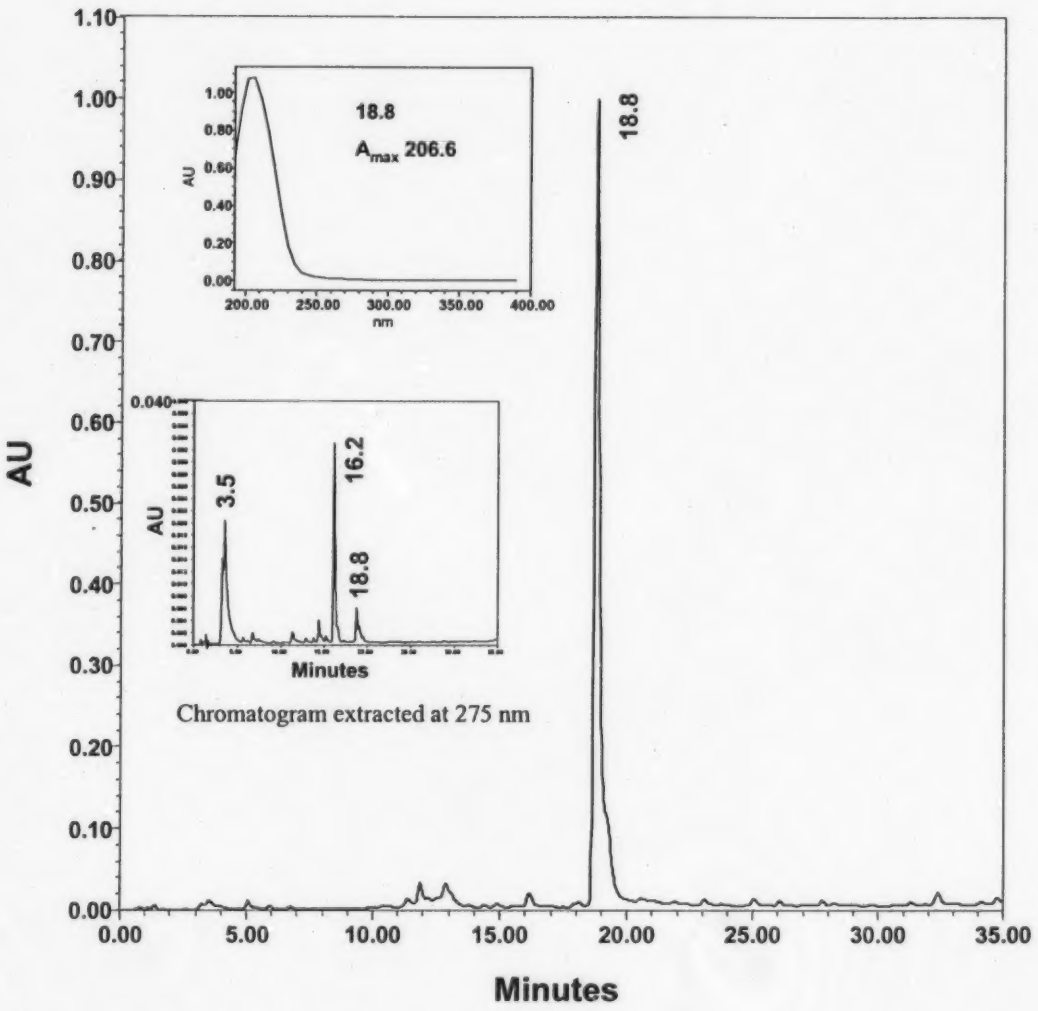


Fig 4. HPLC chromatogram of a bioactive fraction from the chloroform extract of *Xanthium strumarium* fruits at 210 nm.

Spectroscopic data (^{1}HNMR, ^{13}CNMR and FAB-MS) was obtained. Elucidation of the structure is underway.

Selected References

Chemoprevention Working Group. 1999. Prevention of Cancer in the Next Millenium: Report of the Chemoprevention Working Group to the American Association for Cancer Research, **59**, 4743-4758.

Cragg, G., Newman, D. and Snader, K. 1997. Natural Products in Drug Discovery and Development. *J. Nat. Prod.*, **60**, 52-60.

Graham, J., Quinn, M., Fabricant, D. and Farsworth, N. 2000. Plants used against cancer – an extension of the work of Jonathan Hartwell. *J. Ethnopharmacology,* **73**, 347-377.

Hatwell, J. 1968. Plants used against cancer. A survey. *Lloydia*, **31**-2, 122-158.

Hollingshead, M., Alley, M., Camalier, R., Abbott, B., Mayo, J., Malspeis, L. and Grever, M. 1995. In vivo cultivation of tumor cells in hollow fibers. *Life Sci.*, **57**, 131-141.

Huang, K. C. 1999. The Pharmacology of Chinese Herbs. 2nd Ed. CRC Press, Boca Raton, Florida.

Kinghorn, A., Fong, H., Farnsworth, N., Mehta, R., Moon, R., Moriarty, R. and Pezzuto, J. 1998. Cancer Chemopreventive Agents Discovered by Activity-guided Fractionation: A Review. *Curr. Org Chem.*, **2**, 597-612.

Leung, A. Y. 1984. Chinese Herbal Remedies. Universe Books, New York, N.Y.

McMillan, C. 1975a. Experimental hybridization of *Xanthium strumarium* (Compositae) from Asia and America. I. Responses of F1 hybrids to photoperiod and temperature. *Am. J. Bot.*, **62**, 41-47.

McMillan, C., Chavez, P. and Mabry, T. 1975b. Sesquiterpene lactones of *Xanthium strumarium* in a Texas population and in experimental hybrids [Cytogenetics]. *Biochem. Syst. Ecol.* **3**.

Pezzuto, J. 1997a. Plant-Derived Anticancer Agents. *Biochemical Pharm.*, 53, 121-133.

Pezzuto, J. 1997b. Bioassy Methods Useful for Activity-Guided Isolation. In: Chemistry, Biological and Pharmacological Properties of Medicinal Plants from the Americas. Proceedings of the IOCD/CYTED Symposium, Panama Cuty, Panama, Feb. 1997. Ed(s). Hostettman, K., Gupta, M. and Marson, A. Harwood Acad. Pub. 83-110.

Quinn, J. R. 1974. China Medicine As We Saw It". A Publication of the John E. Fogarty International Center for Advanced Study in the Health Sciences. U.S. Department of Health, Education, and Welfare Public Health Service, National Institutes of Health.

Ross, J., Arnason, J. and Birnboim, H. 1999. Low concentrations of the feverfew parthenolide inhibit *in vitro* growth of tumor lines in a cytostatic fashion. *Planta Med.*, 65-2, 126-129.

Roussakis, Ch., Chinou, I., Vayas, C., Harvala, C. and Verbist, J. Cytotoxic Activity of Xanthatin and the Crude Extracts of *Xanthium strumarium*. *Planta Med.*, 60, 473-474.

Tang, W. and Eisenbrand, G. 1992. Chinese Drugs of Plant Origin. Chemistry, Pharmacology, and Use in Traditional and Modern Medicine. Springer-Verlag, Berlin Heidelberg.

D. Personnel

Dr. Yaoge Huang, Postdoctoral Research Fellow, full time over 19 months
Corwin Nycholat, Research Assistant, full time over 2 months
Irving Ramirez, M.Sc. student, full time over 18 months (not funded)
Donna Dunlop, Research Assistant, 10% time over 3 months (not funded)
Dr. Branka Barl, Professional Research Associate, 10% time over 24 months (not funded)
Dr. Robert Hickie, Professor, 3% time over 24 months (not funded)

E. Equipment

None

F. Project Development Material

- Papers in Published Conference Proceedings and Abstracts:

Huang, Y-G., Branka Barl, B. and Ivanochko, G. 2001. Selected Non-Timber Forest Products with Phytomedicinal and Nutraceutical Applications from Jilin Province, China. In: Forest Communities in the Third Millennium: Linking Research, Business, and Policy Toward a Sustainable Non-Timber Forest Product Sector, General Technical Report NC-217 (L. Duchesne, I. Davidson-Hunt, and J. Zasada, compilers); U.S. Department of Agriculture, Forest Service, North Central Research Station, St. Paul, Minnesota, p.88-97.

Ramírez-Erosa, I., Huang, Y-G., Hickie, R.A., Sutherland, R.G. and Barl, B. 2001. *In vitro* cytotoxicity of plant extracts from Saskatchewan wild plants of the family Compositae. In: Book of Abstracts of the 42nd Annual Meeting of the American Society of Pharmacognosy, July 14-18, 2001, Oaxaca, Mexico, p.200.

Huang, Y-G., Ramirez-Erosa, I., Hickie, R.A. and Barl, B. 2000. Study of Saskatchewan wild plants for antitumor activity using knowledge of Traditional Chinese Medicine. In: Proceedings of the International Herb Conference, July 18-21, 2000, Saskatoon. p. 41-43.

- Conference Presentations:

Ramírez-Erosa, I., Huang, Y-G., Hickie, R.A., Sutherland, R.G. and Barl, B. 2001. Extraction of cytotoxic compounds from wild Saskatchewan plants of the family Compositae. The 5th Western Canadian Medicinal and Aromatic Plants Conference, Edmonton, March 5-7, Alberta.

Y. Huang, I. Ramirez-Erosa, R. A. Hickie and B. Barl. 2000. Study of Saskatchewan wild plants for antitumor activity using knowledge of Traditional Chinese Medicine. In: Canadian Cancer Society Annual Meeting, November 18-19, 2000, Saskatoon.

- Herbarium specimens of selected plant species.

G. SDAF Acknowledgment

Same as under F.

H. Expense Statement

Statement of expenditures and receipts has been submitted by the Office of Research Services, University of Saskatchewan.

Appendix A. List of plants with potential anticancer activity collected in the wild throughout Saskatchewan over two years, 1999 and 2000.

No.	Coll. I.D.	Species & Family	English Name & Chinese Name	Date of Harvest & Location	Plant Part & Weight Harvested
1.	058*	*Acorus calamus* Araceae	Sweet flag Chang Pu	Sep. 8 La Ronge#2	Rhizome 542.8g Root 128.4g
2.	040	*Agastache foeniculum* Labiatae	Giant-hyssop Huo Xiang	Aug. 18 U of S	Aerial parts 363.8g
3.	075*	*Alisma plantago-aquatica* Alismaceae	Common water-plantain Xiao Hua Ze Xie	Oct. 8 Hwy 41	Root 290.5g
4.	007	*Allium textile* Liliaceae	Prairie onion Wang Zhuang Cong	June 4 Sa. S. #2	Bulb 0.0g
5.	019	*Anemone canadensis* Ranunculaceae	Canada anemone Jia Na Da Yin Lian Hua	June 17 Hwy #5	All plant 166.4g
6.	001*	*Anemone patens* Ranunculaceae	Crocus anemone Shen Ye Bai Tou Weng	May 5 Bidulph	Rhizome 122.2g.
7.	011	*Apocynum androsaemifolium* Apocynaceae	Spreading dogbane Mei Guo Cha Ye Hua	June 23 La Ronge	All plant 276.1g
8.	003*	*Aralia nudicaulis* Araliaceae	Wild sarsaparilla Luo Jing Cong Mu	June 4 Sa. S.#1	Stem 150.7g Rhiz. 159.5g
9.	068	*Arctostaphylos uva-ursi* Ericaceae	Bearberry Xiong Guo	Sep. 16 Sa. S. #2	Stem and leaf 828.2g
10.	016*	*Artemisia absinthium* Compositae	Absinthe Ni Yin Chen Hao	May 28 Silverspring	Aerial parts 172.6g
11.	009*	*Artemisia campestris* Compositae	Plains wormwood Hong Jing Hao	June 23 La Ronge	All plant 897.5g
12	008*	*Artemisia frigida* Compositae	Pasture sage Leng Hao, Bai Hao	June 17 Silverspring	All plant 529.9g
13.	021*	*Artemisia ludoviciana* Compositae	Prairie sage Lu De Wei Hao	July 20 Sa. S. #2	All plant 239.7g
14.	043*	*Artemisia vulagris* Compositae	Common wormwood Ye Ai (Ai Hao, Ai Ye)	Aug. 19 U of S	Aerial parts 462.9g
15.	069*	*Asparagus officinalis* Liliaceae	Asparagus Shi Diao Bai	Sep. 17 Dona Home	Rhizome 514.2g
16.	072*	*Aster brachyactis* Compositae	Rayless aster	Sep.17 11 st. West	Aerial parts 528.3g
17.	059*	*Aster ciliolatus* Compositae	Lindley's aster Zi Wan	Sep. 8 La Ronge#3	Root 216.8g
18.	014	*Capsella bursa-pastoris* Cruciferae	Shepherd's-purse Ji Cai	June 16 U of S	All plant 201.9g
19.	035*	*Carduus nutans* Compositae	Nodding thistle Fu Chui Fei Ji	July 28 Hwy #219	Aerial parts 500.3g
20.	027*	*Cladina stellaris*	Northern reindeer lichen	June 23 La Ronge	All plant 582.2g
21.	033*	*Cirsium arvense* Compositae	Canada thistle Tian Ji	July 19 U of S	Aerial parts 234.5g
22.	046	*Cornus canadensis* Cornaceae	Bunchberry Yu Shan Ju	Sep.8 La Ronge#1	Mature fruit 176.9g
23.	012	*Crataegus rotundifolia* Rosaceae	Round-leaved hawthorn Shan Zha	Sep 4 Sas. S. #2	Mature fruit 659.2g
24.	060	*Dryopteris austriaca* Polypodiaceae	Spinulose shield fern Kuo Ye Lin Mao Jue	Sep. 9 4#La Ronge	Root 719.7g

Appendix A. List of plants with potential anticancer activity collected in the wild throughout Saskatchewan over two years, 1999 and 2000 *continued.*

No.	Coll. I.D.	Species & Family	English Name & Chinese Name	Date of Harvest & Location	Plant Part & Weight Harvested
25.	074*	*Echinacea angustifolia* Compositae	Purple coneflower Xia Ye Zi Zhui Hua	Oct. 6 U of S	Root 396g
26.	061*	*Epilobium angustifolium* Onagraceae	Fireweed Liu Lan	Sep. 9 La Ronge#7	Root 399.4g
27.	015	*Equisetum arvense* Equisetaceae	Common horsetail Wen Jing	July 20 Sa. S. #2	All plant 196.2g
28.	025*	*Equisetum sylvaticum* Equisetaceae	Woodland horsetail Lin Wen Jing	June 23 La Ronge	All plant 488.0g
29.	066	*Glycyrrhiza lepidota* Leguminosae	Wild licorice Bei Mei Gan Cao	Sep 16 Sa. S. #2	Root 282.5g
30.	020	*Geum triflorum* Rosaceae	Three-flowered avens San Hua Shui Yang Mei	June 17 Silverspring	Root 0.0g
31.	036*	*Helianthus petiolaris* Compositae	Prairie sunflower Ping Yuan Xiang Ri Kui	July 28 Hwy #219	All plant 661.6g
32.	083*	*Inonutus obliquus* Polyporaceae	Chaga, Birch Mushroom Shu She	September 28	Whole mushroom
33.	034	*Juniperus communis* Cupressaceae	Low Juniper Ou Zhou Ci Bai	July 20 Sa. S. #2	Branch 478.7g
34.	022	*Juniperus horizontalis* Cupressaceae	Creeping Juniper Pu Fu Bai	July 20 Sa. S. #2	Branch Needle 933.7g
35.	054	*Ledum groenlandicum* Ericaceae	Common labrador tea Ge Lin La Ba Cha	Sep. 8 La Ronge#1	Branch and leaf 540.3g
36.	042*	*Leonurus cardiaca* Labiatae	Motherwort Ou Yi Mu Cao	Aug. 19 U of S	Aerial parts 374.6g
37.	010*	*Lepidium apetalum* Cruciferae	Common pepper-grass Ting Li Zi, Du Xing Cai	July 20 Sa. S. #2	Seed 210.7g
38.	002*	*Lithospermum incisum* Boraginaceae	Narrow-leaved puccoon Zi Cao	June 3 Patty Ann	Root 62.7g
39.	006	*Lonicera dioica* Caprifoliaceae	Twining honeysuckle Xiao Hua Ren Dong	July 20 Sa. S. #2	Vine with leaf 357.2g
40.	048	*Lycopodium annotinum* Lycopodiaceae	Stiff club-moss Er Nian Shi Song	Sep.8 La Ronge#1	All plant 397.2g
41.	050	*Lycopodium complanatum* Lycopodiaceae	Ground-cedar Bian Ye Shi Song	Sep.8 La Ronge#1	All plant 401.0g
42.	049*	*Lycopodium obscurum* Lycopodiaceae	Ground-pine Yu Bai	Sep. 8 La Ronge#1	All plant 497.4g
43.	071*	*Lythrum salicaria* Lythraceae	Purple loosestrife Qian Qu Cai	Sep. 17 11 st. West	Aerial parts 1018.7g
44.	057	*Maianthemum canadense* Liliaceae	Two-leaved solomon's-seal Jia Na Da Wu He Cao	Sep. 8 La Ronge#1	Mat fruit 143.1g Oth. part 156.7
45.	039	*Malva pusilla* Malvaceae	Round-leaved mallow Jin Kui	Aug. 17 Acadia St	All plant 362.5g
46.	082	*Matricaria matricarioides* Compositae	Pineappleweed	Whole plant September 15	
47.	018*	*Matteuccia struthiopteris* Polypodiaceae	Ostrich fern Xiao Ye Guan Zhong	June 23 La Ronge	Rhizome 805.4g
48.	029*	*Melilotus alba* Leguminosae	White sweet-clover Bai Xiang Cao Mu Xi	July 2 8th Street	Stem: 619.2g Root: 81.7g
49.	030	*Melilotus officinalis* Leguminosae	Yellow sweet-clover Huang Xiang CaoMuXi	July 2 8th Street	Stem: 658.6g Root: 80.5g

Appendix A. List of plants with potential anticancer activity collected in the wild throughout Saskatchewan over two years, 1999 and 2000 *continued.*

No.	Coll. I.D.	Species & Family	English Name & Chinese Name	Date of Harvest & Location	Plant Part & Weight Harvested
50.	024	*Mentha arvensis* Labiatae	Wild mint Bo He	July 10 Wakaw Lake	Aerial parts 144.1g
51.	041*	*Mentha arvensis* Labiatae	Wild mint Bo He	Aug. 18 U of S	Aerial parts 211.2g
52.	073	*Monarda fistulosa* Labiatae	Wild Bergamot Ni Mei Guo Bo He	Oct. 6 U of S	Aerial parts 713g
53.	028	*Morchella elata*	Black morel Yang Du Jun	June 24 P. Albert	Sporophore 119.3g
54.	077	*Phragmites communis* Gramineae	Common reed grass Lu Wei	May 18 Bidulph	Rhizome 162.5g
55.	026	*Pinus banksiana* Pinaceae	Jack pine Bei Mei Duan Ye Song	June 23 La Ronge#1	Branch Needle 650g
56.	051	*Plantago major* Plantaginaceae	Common plantain Da Che Qian	Sep. 8 La Ronge#2	Mature seed 508.2g
57.	076*	*Polygonum amphibium* Polygonaceae	Water smartweed Liang Xi Liao	Oct. 8 Hwy #41	Root 526.8g Ste & lea 548.9
58.	038	*Portulaca oleracea* Portulacaceae	Purslane Ma Chi Xian	Aug. 14 Hwy #11	Aerial parts 0.0g
59.	063	*Potentilla norvegica* Rosaceae	Rough cinquefoil Nuo Wei Wei Ling Cai	Sep. 9 La Ronge#7	Root 292.1g
60.	045	*Prunus virginiana* Rosaceae	Choke cherry Hei Ye Ying Tao	Sep. 4 Sa. S. #2	Mature fruit 1030.3g
61.	067	*Psoralea argophylla* Leguminosae	Silverleaf psoralea Yin Ye Bu Gu Zhi	Sep. 16 Sa. S. #2	Aerial parts 480.0g
62.	032	*Ranunculus cymbalaria* Ranunculaceae	Seaside buttercup Shui Hu Lu Miao	June 30 8th Street	All plant 162 5g
63.	078	*Ranunculus glaberrimus* Ranunculaceae	Shiny-leaved butter cup	May 5 Bidulph	All plant 16.3g
64.	079	*Rosa acicularis* Rosaceae	Prickly rose	July 14 Peggy Ann	Flower bud 59.4g
65.	013	*Rosa arkansana* Rosaceae	Low prairie rose A Ken Se Qiang Wei	June 14, Sa. S. #2	Flower 36.9g
66.	005*	*Rhus radicans* Anacardiaceae	Poison-ivy Qi Gen Du Teng	June 4 Sa. S. #1	Root 148.7g
67.	031*	*Rumex fennicus* Polygonaceae	Field dock Hong Niu Er Da Huang	July 2 8th Street	Root 427.5g
68.	044	*Rumex stenophyllus* Polygonaceae	Narrow-leaved field dock Zhai Ye Suan Mo	Aug. 12, Hwy #5	Root 174.9g
69.	062	*Scutellaria galericulata* Labiatae	Marsh skullcap Kui Zhuang Huang Qin	Sep. 8 La Ronge#2	All plant 237.7+36.9g
70.	053	*Scirpus lacustris* Cyperaceae	Common great bulrush Shui Cong	Sep. 8 La Ronge#3	All plant 424.3g
71.	023*	*Senecio vulgaris* Compositae	Common groundsel Ou Zhou Qian Li Guang	June 23 La Ronge	All plant 285.1g
72.	081	*Solidago canadenesis* Compositae	Graceful goldenrood J N Da Yi Zhi Huang Hua	July 28 Hwy #219	All plant 0.0g
73.	037*	*Solidago missouriensis* Compositae	Low goldenrod Yi Zhi Huang Hua	July 28 Hwy #219	All plant 694.6g

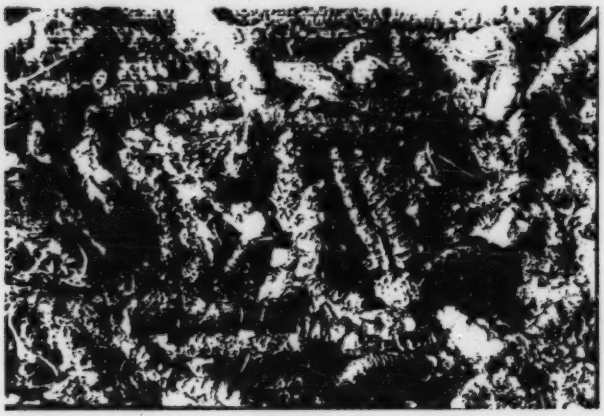
Appendix A. List of plants with potential anticancer activity collected in the wild throughout Saskatchewan over two years, 1999 and 2000 *continued.*

No.	Coll. I.D.	Species & Family	English Name & Chinese Name	Date of Harvest & Location	Plant Part & Weight Harvested
74.	064*	*Solidago mollis* Compositae	Velvety goldenrod Yi Zhi Huang Hua	Sep. 3 Silverspring	All plant 526.7g
75.	080	*Solidago rigida* Compositae	Stiff goldenrod Jian Yeng YiZhi Hua Hua	Sep. 3 Silverspring	All plant 0.0g
76.	065*	*Sonchus arvensis* Compositae	Perennial sow-thistle Qu Mai Cai	Sep. 16 Diefenbak P.	All plant 415.8g
77.	055	*Stellaria media* Caryophyllaceae	Common chichweed Fan Lu (Chai Hu)	Sep. 8 La Ronge#4	All plant 167.3g
78.	070*	*Tanacetum vulgare* Compositae	Tansy Ju Hao (Ai Ju)	Sep. 17 Silverspring	Aeriel parts 738.1g
79.	017*	*Taraxacum officinale* Compositae	Dandelion Ou Zhou Pu Gong Ying	May 27 Outlook	Aerial p 310.0g; Root 119.3g.
80.	004*	*Thalictrum venulosum* Ranunculaceae	Veiny meadow-rue Wang Zh. Tang Song Cao	June 14 Sa. S. #2	Root: 121.1g; Aerial: 121.8g
81.	056	*Trifolium hybridum* Leguminosae	Alsike clover Za Zhong Che Zhou Cao	Sep. 8 La Ronge#2	All plant 536.0g
82.	052	*Typha latifolia* Typhaceae	Common cattail Kuan Ye Xaing Pu	Sep. 9 #4 La Ronge	Pollen 2420.5g
83.	047*	*Xanthium strumarium.* Compositae	Cocklebur Cang Er	Sep.4 Iron Bridge	Mature fruit 396.8g

*Plants tested for anticancer activity

Appendix B. Images of 40 plant species screened for anticancer activity *in vitro.*

Acorus calamus (Sweet flag)



Alisma plantago-aquatica
(Commom water-plantain)

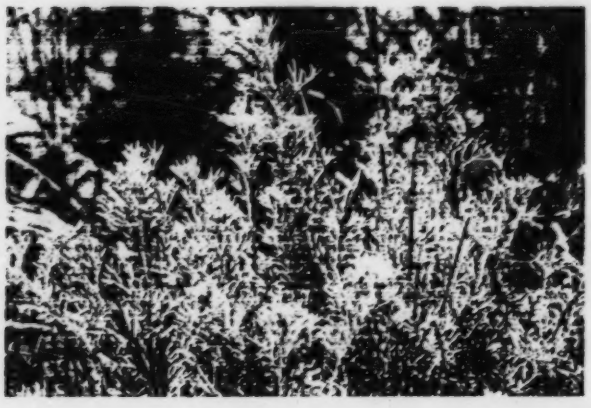
Anemone patens (Crocus anemone)

Aralia nudicaulis (Wild sarsaparilla)

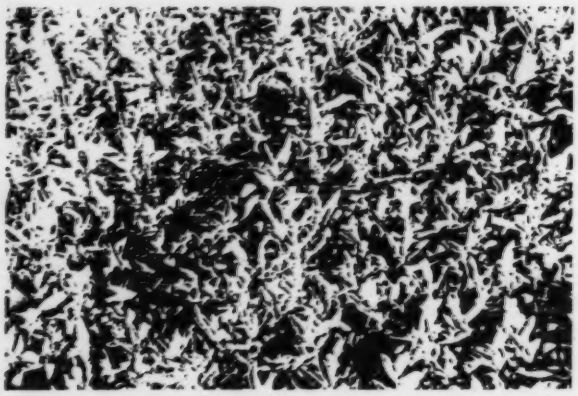
Artemisia absinthium (Absinthe)

Artemisia campestris
(Plains wormwood)

Artemisia frigida (Pasture sage)



Artemisia ludoviciana
(Prairie sage)



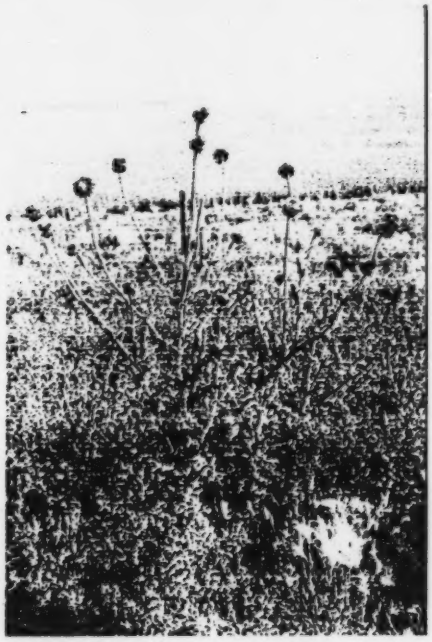
Artemisia vulgaris
(Common wormwood)

Asparagus officinalis (Asparagus)

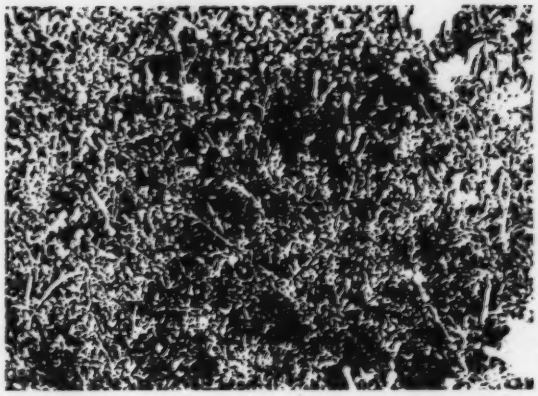
Aster brachyactis (Rayless aster)

Aster ciliolatus (Lindley's aster)

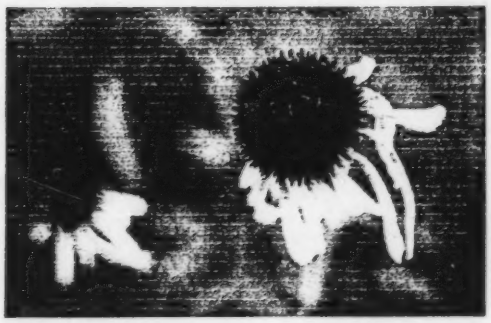
Carduus nutans (Nodding thistle)



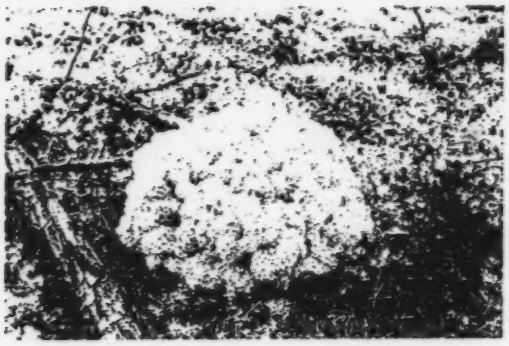
Cirsium arvense (Canada thistle)



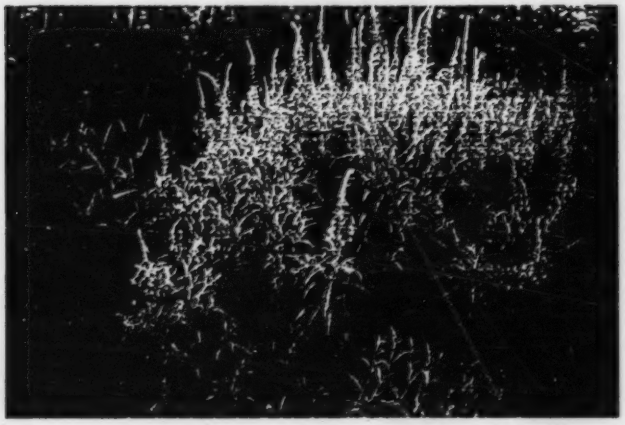
Echinacea angustifolia
(Narrow-leaved purple coneflower)



Cladina stellaris
(Northern reindeer lichen)



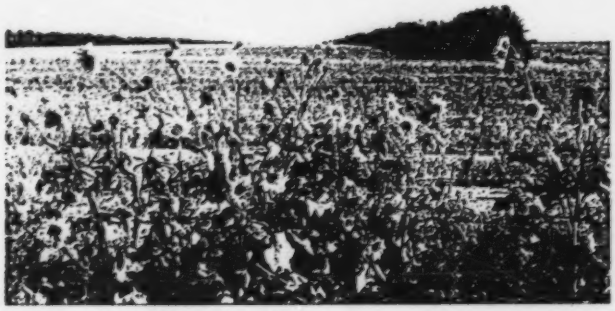
Epilobium angustifolium (Fireweed)



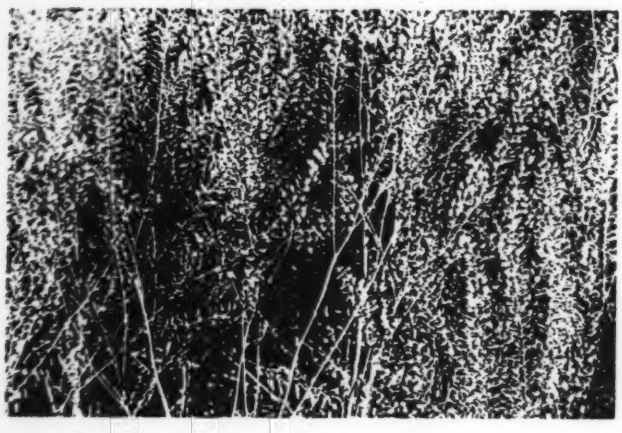
Equisetum sylvaticum
(Woodland horsetail)

Leonorus cardiaca (Motherwort)

Helianthus petiolaris (Prairie sunflower)



Lepidium apetalum
(Common pepper-grass)

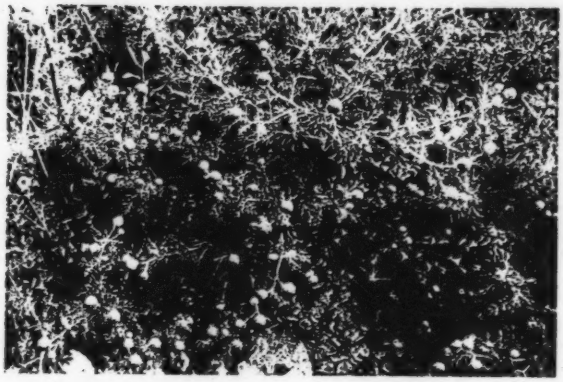


Lithospermum incisum
(Narrow-leaved puccoon)

Lycopodium obscurum (Ground-pine)

Lythrum salicaria (Purple loosestrife)

Matricaria matricarioides
(Pineappleweed)

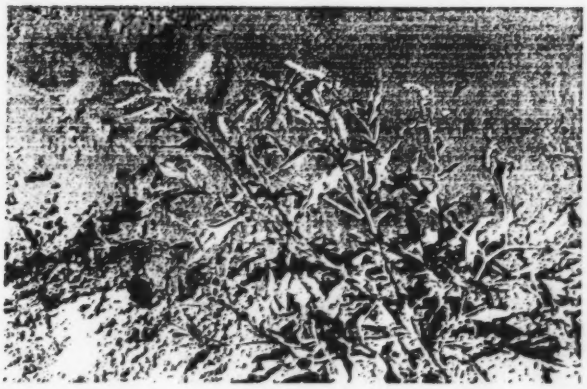


Matteuccia struthiopteris (Ostrich fern)

Melilotus alba (White sweet-clover)

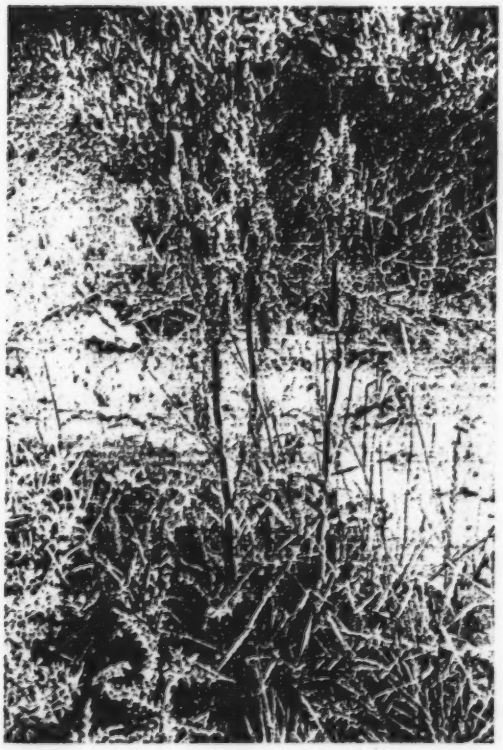
Mentha arvensis (Wild mint)

Polygonum amphibium
(Water smartweed)



Rhus radicans (Poison-ivy)

Rumex fennicus (Field dock)

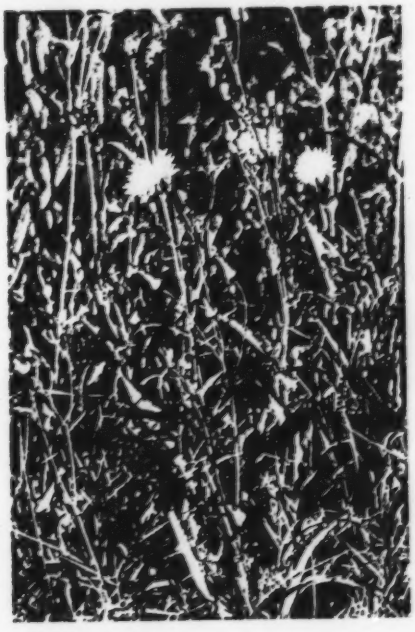


Senecio vulgaris (Common groundsel)

Solidago missouriensis (Low goldenrod)

Solidago mollis (Velvety goldenrod)

Sonchus arvensis (Perennial sow-thistle)

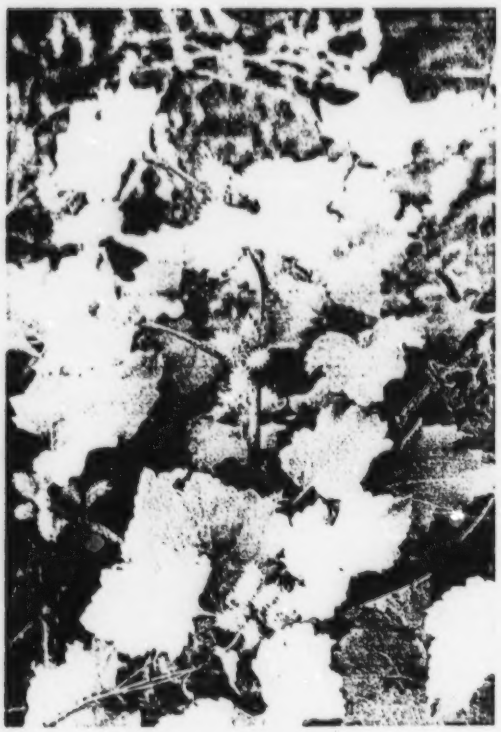


Tanacetum vulgare (Tansy)

Taraxacum officinale (Dandelion)

Thalictrum venulosum
(Veiny meadow-rue)

Xanthium strumarium (Cocklebur)



Mushroom "Shu She"